Neuroendocrine biomarkers in acute stroke

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Zusammenfassung der:

Habilitationsschrift

"Neuroendocrine biomarkers in acute stroke"

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vorgelegt von

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Summary:

Each year, over 5 million people die as a consequence of stroke worldwide, and at least 1 in 6 patients who survive a stroke will suffer another stroke within 5 years[1]. Yet, even validated clinical prognostic models are not sufficiently precise to predict outcome in patients with stroke[2]. In this context blood biomarkers may add to improve prognostic accuracy for risk stratification, decision-making and thus ultimately patient outcome. Our research focus was to identify and assess different blood biomarkers to improve risk stratification and identification of stroke etiology. We selected the most promising neuroendocrine candidate biomarkers.

During stress, vasopressin is a potent synergistic factor of corticotropin releasing hormone (CRH) as a hypothalamic stimulator of the hypothalamo-pituitary-adrenal (HPA)- axis[3-7]. Measurements of CRH and vasopressin levels are cumbersome because of their instability and short half-life[8-10]. In contrast, copeptin is a more stable peptide, which is stoichiometrically released from the vasopressin precursor molecule[11].

The aim of our first study was to evaluate copeptin as a new “stress marker” and to compare it to cortisol levels in different stress situations (i.e. healthy controls, medical patients and patients after heart surgery). Copeptin showed a more pronounced increase upon substantial physical stress as compared to cortisol levels. It reflected even moderate stress more subtly as compared to cortisol. Thus, copeptin appeared to be a new promising stress marker (study I)[12].

After identifying copeptin as a stress biomarker, we determined its value as a prognostic marker in patients with acute ischemic stroke. In this cohort study, copeptin was measured on admission in consecutive stroke patients and neurological outcome was assessed at 3 months. Copeptin remained highly predictive even after adjusting for risk factors and it improved the prognostic accuracy of the established National Institute of Health Stroke Score (NIHSS). Thus, we concluded that copeptin is a novel, independent prognostic marker improving currently used risk stratification of stroke patients (study II)[13].

The predictive value of copeptin persisted also when assessed for long-term functional outcome and mortality one year after the index stroke (study III)[14].

In the same stroke cohort we evaluated the prognostic value of cortisol, triiodothyronine (T3), free thyroxine (fT4), thyroid- stimulating hormone (TSH) and growth hormone (GH) simultaneously. We found that the prognostic accuracy of cortisol was higher compared to the other anterior pituitary axis hormones. Cortisol was an independent prognostic marker of functional outcome and death within 90 days and one year but, unlike copeptin, cortisol contributed only limited additional predictive value to the NIHSS score (study IV)[15].

To validate the incremental value of copeptin in the prediction of outcome compared to established clinical variables, we performed an independent multicenter cohort study. We demonstrated that in patients with ischemic stroke, copeptin is the first validated blood biomarker that added predictive information for functional outcome and mortality at three months beyond established prognostic models including, vascular risk factors, age, comorbidities, imaging

information and the NIHSS. In addition, copeptin also showed to be a new promising blood biomarker for prediction of in-hospital complications (study V)[16].

As copeptin was an accurate prognostic marker in acute ischemic stroke we aimed at assessing its reliability for risk stratification in patients with transient ischemic attacks (TIAs). We thus conducted a prospective study in TIA patients. Copeptin but not cortisol improved the prognostic accuracy of the routinely used ABCD2 risk score. We concluded that if validated in an independent cohort, routine copeptin measurement may be an additional tool for risk stratification and targeted resource allocation after TIA (study VI)[17].

Besides their application as a prognostic tool, serum biomarkers may also enhance etiologic classification and thus improve patient allocation to the appropriate therapy. A-type natriuretic peptides (ANP) have been associated with the activation of the sympathetic nervous system and have been proposed as biomarkers of atrial fibrillation (AF)[18,19]. So we assessed the diagnostic value of mid-regional pro ANP in acute ischemic stroke patients and found that it was a new independent and reliable diagnostic marker for cardioembolic etiology (study VII)[20].

Early predictors for the development of stroke-associated infection may identify high-risk patients and reduce post-stroke infection and mortality. We found that among ischemic stroke patients, copeptin, procalcitonin, white blood cell count and CRP measured on admission were predictors of infection within 5 days after stroke. The combination of these biomarkers improved the prediction of patients who developed an infection (study VIII)[21].

This cumulative habilitation thesis is based on the following publications:

Incorporating only first or last authorships.

I. **Mira Katan**, Nils Morgenthaler, Isabell Widmer, Jardena Puder, Caroline Koenig, Beat Mueller, Mirjam Christ-Crain. „Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level". Neuro Endocrinol Lett. 2008; 29(3): 341-346.

II. **Mira Katan**, Felix Fluri, Nils Morgenthaler, Philipp Schuetz, Christian Zweifel, Roland Bingisser, Klaus Mueller, Stefan Meckel, Achim Gass, Ludwig Kappos, Andreas Steck, Stefan Engelter, Beat Mueller, Mirjam Christ-Crain. "Copeptin, the C-terminal part of the Vasopressin pro-hormone to predict outcome in patients with stroke". Annals of Neurology. 2009; 66(6): 799-808.

III. Sandrine Urwyler, Philipp Schuetz, Felix Fluri, Nils G. Morgenthaler, Christian Zweifel, Andreas Bergmann, Roland Bingisser, Ludwig Kappos, Andreas Steck, Stefan Engelter, Beat Mueller, Mirjam Christ-Crain, **Mira Katan.** "Prognostic value of copeptin: one-year outcome in patients with acute stroke". Stroke. 2010; 41(7): 1564-1567.

IV. Stefanie Neidert***, Mira Katan*,** Felix Fluri, Nils G. Morgenthaler, Christian Zweifel, Andreas Bergmann, Roland Bingisser, Ludwig Kappos, Andreas Steck, Stefan Engelter, Beat Mueller, Mirjam Christ-Crain "Anterior pituitary-axis-hormones and outcome in acute ischemic stroke". J Intern Med. 2011; 269(4): 420-432.

V. GianMarco De Marchis***, Mira Katan*,** Anja Weck, Felix Fluri, Christian Foerch, Oliver Findling, Philipp Schuetz, Daniela Buhl, Marleen Seiler, Nils G Morgenthaler, Heinrich P Mattle, Beat Mueller, Mirjam Christ-Crain, Marcel Arnold. "Copeptin adds prognostic information after ischemic stroke: Results from the CoRisk Study". Neurology. 2013; March 6 (Epub ahead of print).

* Equally contributing first author

VI. **Mira Katan,** Nicole Nigro, Felix Fluri, Nils Morgenthaler, , Philipp Schuetz, Stefanie Neider, Felix Jax, Stefan Meckel, Achim Gass, Ludwig Kappos, Andreas Steck, Stefan Engelter, Beat Mueller, Mirjam Christ-Crain. "Stress Hormones to predict cerebrovascular Re-events in patients with transient ischemic attacks". Neurology. 2011; 76(6): 563-566.

VII. **Mira Katan**, Felix Fluri, Philipp Schuetz, Nils Morgenthaler, Christian Zweifel, Roland Bingisser, Ludwig Kappos, Andreas Steck, Stefan Engelter, Beat Mueller, Mirjam Christ-Crain. „Midregional pro-atrial natriuretic peptide in patients with acute ischemic stroke". J Am Coll Cardiol. 2010; 56(13): 1045-1053.

VIII. Felix Fluri, Nils Morgenthaler,, Beat Mueller , Mirjam Christ-Crain, **Mira Katan**. "Copeptin, Procalcitonin and routine inflammatory markers-predictors of infection after stroke". PLoS One. 2012; 7(10): e48309.

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List of abbreviations

ABCD2	A=age, B=blood pressure, C=clinical manifestation, D=duration, D=diabetes; items of risk stratification score
ACTH	AdrenoCorticoTropic Hormone
AF	Atrial Fibrillation
AUC	Area Under the Curve
AVP	Arginin- Vasopressin
BNP	Brain Natriuretic Peptide
CE	CardioEmbolic
CCI	Charlson comorbidity Index
95%CI	95% Confidence Intervall
CoRisk	Copeptin for Risk stratification study
CRH	Corticotropin-Releasing Hormone
CRP	C- Reactive Protein
CT	Computed Tomography
DWI	Diffusion Weighted Imaging
ECG	ElectroCardioGram
fT4	free Thyroxine
GH	Growth Hormone
HPA- axis	Hypothalamo- Pituitary- Adrenal- axis
IQR	InterQuartile Range
LACS	Lacunar Syndrome
MRI	Magnetic Resonance Imaging
MRproANP	Midregional part of the prohormone of the Atrial Natriuretic Peptdie
mRS	modified Rankin Scale
Myct	Monocytes
NIHSS	National Institute of Health Stroke Scale
NRI	Net Reclassification Index
NT-BNP	N- Terminal part of the Brain Natriuretic Peptide
OI	Other Infections
OR	Odds Ratio
PACS	Partial Anterior Circulation Syndrome
PCT	ProCalciTonin
POCS	Posterior Circulation Syndrome
T3	Triiodothyronine
TACS	Total Anterior Circulation Syndrome
TIA	Transient Ischemic Attack
TOAST	Trial of Org 10172 in Acute Stroke Treatment
TSH	Thyroid Stimulating Hormone
UTI	Urinary Tract Infections
WBC	White Blood Cells

1. Introduction

1.1. Prognosis after acute ischemic stroke

Stroke is the third leading cause of death and the leading cause of chronic serious disability[22]. Each year, more than 5 million people die as a consequence of stroke, and at least 1 in 6 patients who survive a stroke will suffer another stroke within 5 years[1]. Thus many patients fear the disabling consequences of stroke even more than those of coronary heart disease. However, stroke risk factors and outcome predictors have generally received less attention. Thus, the development of a credible evidence base of prognostic information for outcomes that are meaningful to patients and clinicians, including level of independency, is required. However, even currently available, validated clinical prognostic models are still in need for further improvement[2]. In this context blood biomarkers may add to improve prognostic accuracy for risk stratification, decision-making and thus ultimately patient outcome.

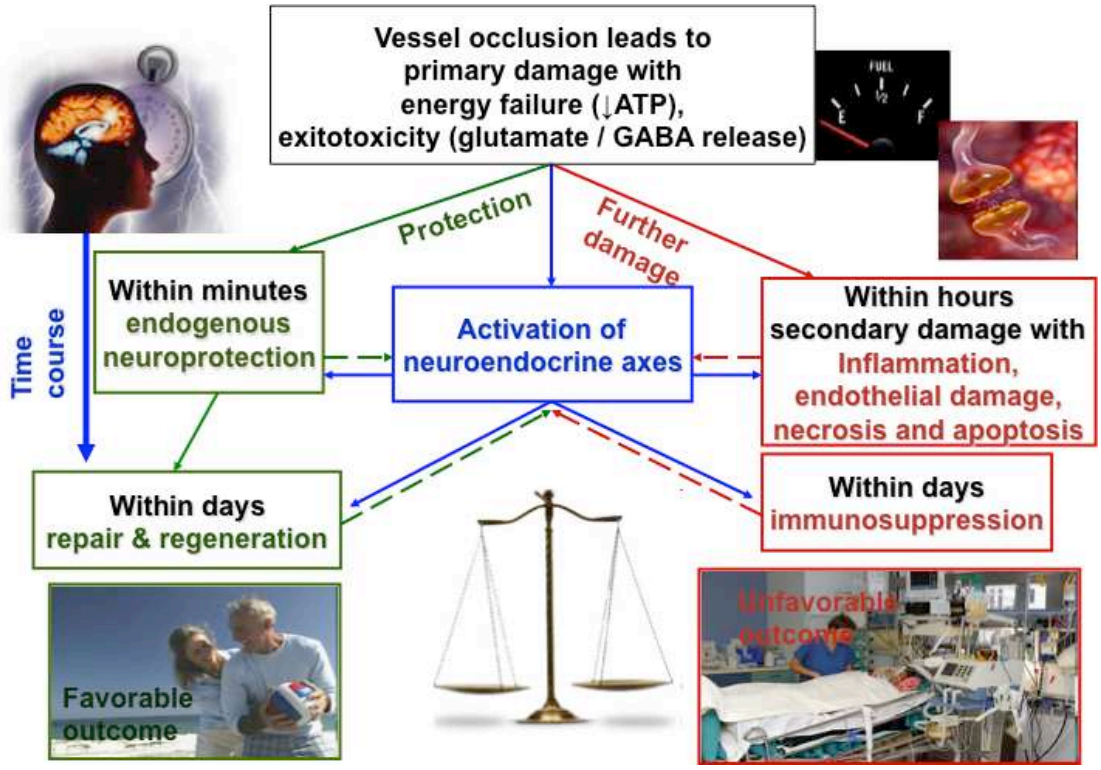
1.2. Blood biomarkers in the setting of stroke

A blood biomarker in the setting of acute stroke can be any quantifiable entity that assesses the manifestation of a stroke-related process. There may be markers for example to guide risk stratification, to characterize stroke size and clinical severity, to reveal stroke etiology, to select appropriate treatment options, to identify patients that may benefit most from interventions, to monitor treatment efficacy, and to recognize the risk of worsening or complications in the short term or the risk of unfavorable long term outcome. To address these questions,

stroke-related biomarkers need to be associated with diverse pathophysiological processes immediately preceding stroke or processes during and after stroke.

Brain injury following transient or permanent focal cerebral ischemia results from a complex cascade of pathophysiological events that evolve in time and space[23]. In ischemic stroke the inciting event is the embolic or thrombotic obstruction of arterial flow to the brain. In brief, sudden cerebral hypoperfusion leads to cellular bioenergetic failure, excitotoxicity, and oxidative stress with subsequent blood brain barrier dysfunction, inflammation, perturbation of haemostasis, activation of neuroendocrine axes, and eventually apoptosis and necrosis of neuronal, glial and endothelial cells[23-27]. Outcome after ischemic stroke depends on all these factors, which contribute to break the balance either towards a favorable or unfavorable outcome (figure 1).

Figure 1. Ischemic cascade and Prognosis after stroke (slide by Mira Katan 2009)



1.3. Implementation and selection of stroke biomarkers

The most fruitful implementation for stroke biomarkers is in areas where information from traditional clinical sources is currently limited such as:

A) Risk stratification and prediction of adverse outcomes since they are prerequisites for safe decision-making for interventions, the degree of monitoring (intensive care unit, intermediate care or normal ward), the need for hospitalization versus outpatient management.

B) Determination of stroke etiology, since this has also direct implications for treatment decisions.

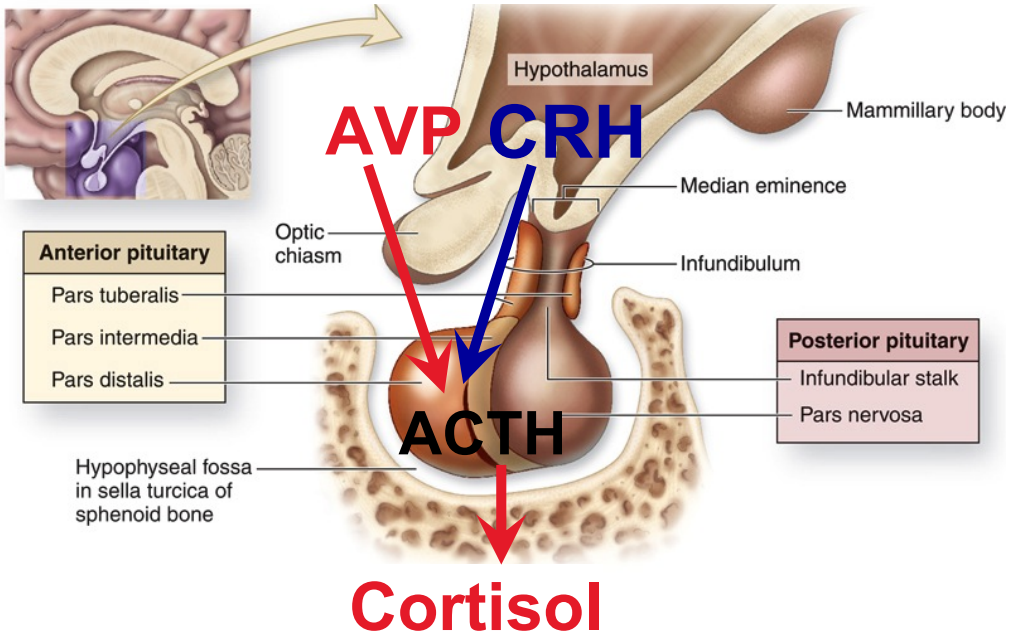
Our main research focus over the past 5 years was to identify and assess different blood biomarkers to improve risk stratification and the identification of stroke etiology. Instead of picking the largely investigated brain tissue or coagulation markers such as astroglial S100beta or d-dimeres, we chose an innovative, more holistic approach and selected the most promising candidate biomarkers of the neuroendocrine system. Activation of the hypothalamo-pituitary-adrenal- axis or “stress- axis” is among the first measurable physiological response to cerebral ischemia[28-31]. Neuroendocrine markers act and interact to preserve overall homeostasis after acute tissue damage. Each “stressor” such as the impact of neuronal cell death or the impact of comorbidities, even social factors, influences the final “stress” marker level. As such they reflect the ‘global threat’ to the body and thus they may indicate when the threshold to return to homeostasis is crossed and the outcome of the individual shifts towards an unfavorable outcome. Besides the advantage of

incorporating information from different systems, acting and interacting systemically as well as within the brain, some of these neuroendocrine markers are easily accessible and can be measured reliably.

1.4. Neuroendocrine biomarkers

The hormonal cascade initiated by a "stressor" through brain stem and limbic pathways involves the release of CRH from parvocellular neurons in the paraventricular nucleus of the hypothalamus[32]. CRH stimulates the release of ACTH from the anterior pituitary gland. Another hypothalamic hormone, which is stimulated by different stressors and probably different pathways, is arginine-vasopressin. AVP seems to exert a potentiating action on CRH and these two agents together are considered the main secretagogues of ACTH[3-7] (figure 2).

Figure 2. Main secretagogues of ACTH

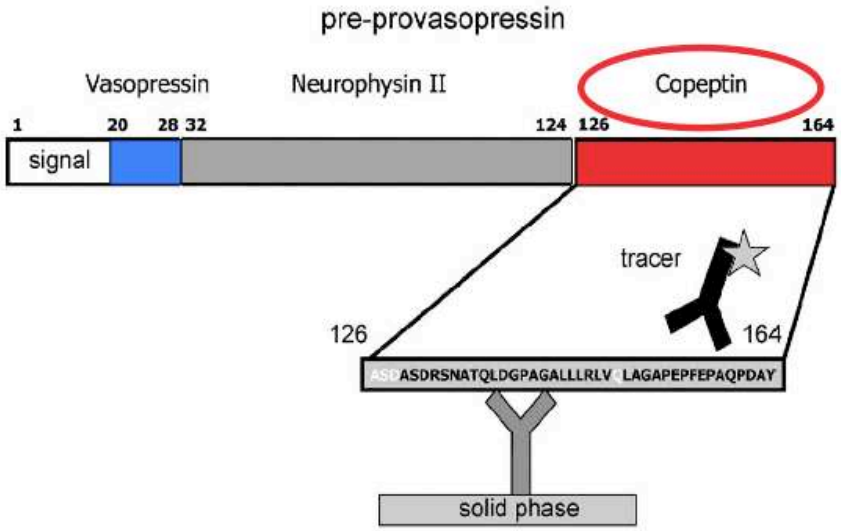


Adapted from Katan et al. Swiss Med Wkly. 2010[33]

ACTH, in turn, stimulates the adrenal cortex to produce cortisol. Cortisol is the "classical" stress hormone at a peripheral level and easy to measure. However, the assessment and interpretation of cortisol levels to assess the integrity of the HPA-axis is dependent on an intact anterior pituitary and adrenal gland. For a direct assessment of recognition of a stressor at the level of the central nervous system, the measurement of CRH or AVP has theoretical advantages as these hypothalamic hormones are produced in the brain but are directly released in the systemic circulation. They may, therefore, be optimal blood biomarkers for several neurological illnesses since they do not require blood brain barrier disruption to be systemically measurable. Thus they can be considered directly reflective of brain disease activity even without major destruction. However, the measurement of circulating CRH or AVP levels is challenging. Both CRH and AVP are released in a pulsatile pattern, are unstable (especially at room temperature), and are rapidly cleared from plasma within minutes[8-10,34.] AVP derives from a larger precursor peptide (pre-provasopressin) along with two other peptides, neurophysin II and copeptin. Copeptin is released in an equimolar ratio to AVP and is more stable in the circulation and easy to measure. Copeptin levels closely mirror the production of AVP[34] (figure 3).

We hence believed that copeptin may reflect the individual "stress-burden" or degree of disruption of "homeostasis" on a hypothalamic level while elegantly bypassing the blood brain barrier due to direct systemic release.

Figure 3. Pre-provasopressin



adapted from Struck J, Morgenthaler NG, Bergmann A. Peptides 2005, 26:2500-2504

2. Methods and Results

2.1. Evaluation of Copeptin as a new neuroendocrine biomarker

Based on the fact that AVP is involved in the stress response and based on the findings that copeptin mirrors AVP levels we first evaluated copeptin under the influence of different physical stressors. To evaluate the discriminatory value of copeptin as compared to cortisol and to assess different stress-levels we performed an observational study including patients with presumed increasing levels of stress. We hypothesized that copeptin as a brain-derived stress hormone provides a more direct and subtle measure of the individual stress level compared to cortisol.

2.1.1. Study I

To gather pilot data[12] we intended to test three categories (group A-C) of patients, all without prior evidence for HPA- axis dysfunction, in different levels of stress caused by the severity of the underlying illness. Ethical approval was obtained, and the patients or their legal representatives gave written informed consent. Group A (no stress) contained 20 healthy control subjects, without apparent stress. Group B (moderate stress) consisted of 25 patients consecutively recruited from a wide and representative variety of patients having been hospitalized on the medical ward for at least 48 hours. In Group C, 29 stable surgical patients were consecutively recruited undergoing elective coronary bypass grafting under general anaesthesia. Peak cortisol levels are achieved in the immediate postoperative period, around the time of extubation[35,36]. Therefore, large surgery can serve as a standardized physiological model for studying major stress[37]. Indeed, copeptin showed a gradual increase with increasing levels of stress and, in contrast to cortisol levels, differentiated between healthy control subjects without apparent stress and medical patients with a moderate degree of stress[12,32]. In addition, copeptin showed a more pronounced increase upon major stress as compared to cortisol (the increase in cortisol was 265 ± 34% and in copeptin 1430 ± 157%, $p<0.001$)[12].

2.2. Neuroendocrine biomarkers for the prediction of functional outcome and mortality in stroke patients

Many publications have reported an association of different biomarkers (e.g. C reactive protein, matrix metalloproteinase 9, neuron specific enolase) with stroke severity or outcome. However, none of these publications reported that the analyzed biomarker provided additional prognostic information compared to established clinical variables (e.g. age, blood pressure, nicotine, dyslipidemia, co-morbidities such as heart failure and lesion size) or whether the biomarker increased the predictive power of validated clinical prognostic scores[38] such as the NIHSS Score. We thus conducted a prospective cohort study to assess the incremental value of copeptin in the prognosis after stroke and TIA. The study was approved by the local ethics committee. From 605 screened patients with suspected cerebrovascular events, in 362 patients an ischemic stroke, in 40 patients a hemorrhagic stroke and in 107 patients a TIA was diagnosed according to the World Health Organization Criteria[39] . At baseline we recorded vital signs and vascular risk factors. Co-morbidities were assessed by the modified Charlson Comorbidity Index[40]. Severity of stroke was determined on admission by the NIHSS[41]. In TIA patients we recorded the ABCD2 Score. Computed tomography or magnetic resonance imaging was performed on admission on each patient. Detailed information such as cardiac and neurovascular ultrasound and 24-hour ECGs were collected to define stroke etiology according to the TOAST classification[42]. The clinical stroke syndrome was determined by applying the criteria of the Oxfordshire Community Stroke

Project; i.e. total anterior circulation syndrome, partial anterior circulation syndrome, lacunar syndrome, and posterior circulation syndrome[43]. Blood samples were collected within 72 h hours from symptom onset and categorized into 0-3h (window for thrombolysis at the time the study was conducted); 3-12h; 12-24h and 24-72h. Plasma was frozen at – 70 °C. Copeptin measurement was done in a single batch with a commercial sandwich immunoluminometric assay with a lower detection limit of 0.4 pmol/L and a functional assay sensitivity (< 20 % inter assay coefficient of variance) of < 1 pmol/L[44].

2.2.1. Study II

The primary end point of this study[13] was unfavorable functional outcome after 90 days from baseline, defined as modified Rankin Scale of 2 to 6 points. The secondary end point was death from any cause within 90-days follow-up[13].

In this prospective, observational study, the median age of patients with ischemic stroke was 75 (IQR 63-83) years and 41% were women. An unfavorable functional outcome was found in 151 patients (42%) with a median mRS score of 4 (IQR 3-6). Forty-four patients died and mortality rate was thus 12%. We found copeptin to be a novel, strong, and independent prognostic marker for functional outcome and death (table 1).

Table 1. Multivariate analyses for the primary and secondary outcome

Multivariate analysis for functional outcome				
Predictor	**Odds ratio**	**(95% CI*)**		***P-value***
Copeptin (increase per log unit) *	2.57	1.27	5.17	**0.01**
Age (increase per unit)	1.06	1.04	1.09	***<0.0001***
Female sex	1.43	0.82	2.49	0.21
Stroke severity, NIHSS (increase per unit)	1.17	1.10	1.23	***<0.0001***
Charlson Index (increase per unit)	1.31	1.09	1.58	***0.004***
TACS	1.51	0.55	4.15	0.42

Multivariate analysis for mortality				
Predictor	**Odds ratio**	**(95% CI*)**		***P-value***
Copeptin (increase per log unit)	4.31	1.65	11.25	***0.003***
Age (increase per unit)	1.07	1.03	1.12	***0.002***
Stroke severity, NIHSS (increase per unit)	1.16	1.09	1.23	***<0.0001***
TACS	1.52	0.51	4.55	0.458

* Increase per log unit-> increase per 1 unit = increase in 10pmol/L

adapted from M.Katan et al., Ann Neurol 2009

In addition, copeptin had a superior discriminatory value if compared to CRP (AUC 0.61; 95% CI 0.55-0.68; p<0.001), white blood cell count (AUC 0.55; 95% CI 0.49-0.62; p<0.0001) and glucose (AUC 0.57; 95% CI 0.50-0.63; p<0.001). Importantly, copeptin improved the prognostic accuracy of the NIHSS score. The combination of the clinical score with the biomarker revealed a significantly higher AUC of 0.79 (95% CI 0.75-0.84) to predict functional outcome if compared to the clinical score or the marker alone. The combined AUC of 0.89 (95% CI 0.84-0.94) predicting death was even higher. To further estimate the incremental prognostic information of copeptin levels to the traditional outcome predictor (i.e. NIHSS), we calculated the net reclassification improvement, which has recently been proposed as a statistical method to evaluate prognostic biomarkers[45]. This statistic examines whether the addition of a blood marker moves those with unfavorable outcome to higher risk categories more often than to lower risk

categories and those with a favorable outcome to lower risk categories more often than to higher risk categories. We used a priori risk groups based on risk classification by Adams et al. and Goldstein et al.[46,47]. The NRI for functional outcome was 39% and for mortality 48% thus copeptin helped in 39 %, respectively 48% of patients to improve classification if compared to the NIHSS alone. Further, time to death was analyzed by Kaplan Meier survival curves. Patients in the lower two quartiles had a minimal risk of death, in contrast to patients with copeptin levels in the 3rd and 4th quartile ($p<0.0001$).

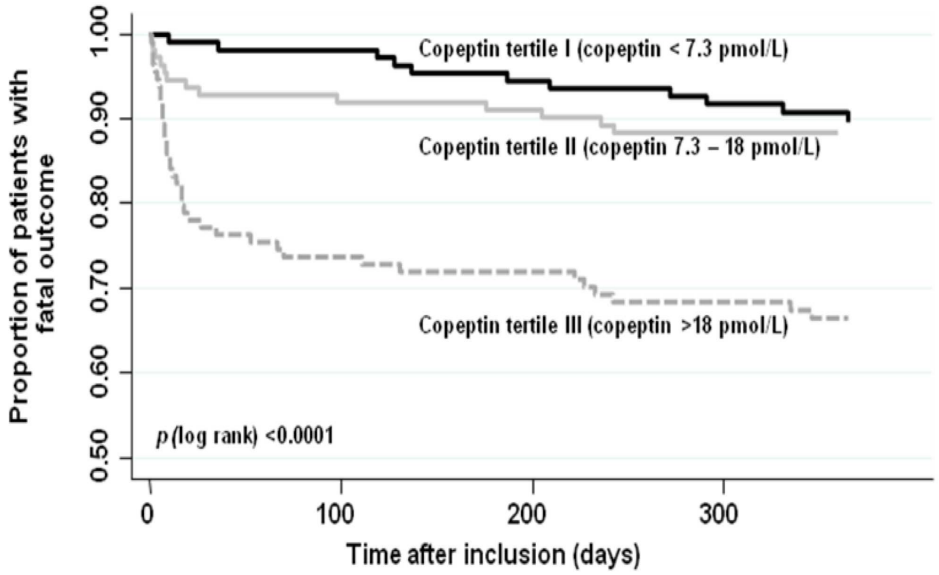
To strengthen the assumption that copeptin is an accurate marker in the very acute phase we analysed the subgroup of patients with symptom onset between 0–3 hours (n = 78) and the prognostic accuracy for functional outcome and mortality was very similar to the overall sample (data not shown).

2.2.2. Study III

Next we performed a long-term follow-up study[14] in the aforementioned cohort aiming to evaluate copeptin levels as a potential marker to predict long-term functional outcome and mortality in acute stroke patients one year after admission to the emergency department[14]. From 362 patients, 341 (94.2%) completed the one-year follow-up. Multivariate logistic regression analysis adjusted for age and NIHSS showed that copeptin was an independent predictor for functional outcome (OR 4.00, 95%CI 1.94-8.19) and death (OR 2.68, 95%CI 1.24-5.82). The AUC of copeptin for functional outcome was 0.72 (95%CI 0.67-0.77) and for mortality 0.74 (95%CI 0.69-0.78). Copeptin levels significantly improved the AUC of NIHSS for functional outcome from 0.70 (95%CI 0.64-0.74)

to 0.76 (95%CI 0.71-0.82, p=0.002) and for mortality from 0.74 (95%CI 0.69-0.78) to 0.78 (95%CI 0.71-0.85, p=0.04). In addition we calculated Kaplan Meier curves and stratified patients based on copeptin tertiles. As demonstrated in Figure 4, we found an increased risk for mortality with increasing copeptin tertiles, particularly from the second to the third tertile.

Figure 4. Copeptin and stroke mortality



Number at risk				
Copeptin tertile I	109	107	103	100
Copeptin tertile II	113	104	103	100
Copeptin tertile III	114	84	82	78

Urwyler S. et al. Stroke 2010.[14]

2.2.3. Study IV

To assess if other markers of the anterior pituitary axis hormones namely cortisol, T3, fT4, TSH and GH are associated with functional outcome and mortality within 90 days and one year after stroke we measured these hormones

in blood samples of the same stroke cohort which were obtained from an indwelling venous catheter the first morning after admission. For this study[15] blood was collected at 7am the day after admission for standardized measurement of hormones. Hormone measurements on day one were not available in the whole cohort, either because patients had died or were already discharged on day one thus for the final analysis 281 ischemic stroke patients were included. Cortisol was measured with a competitive chemiluminescence immunoassay (IMMULITE 2000; Siemens Medical Solution Diagnostics, Los Angeles, CA, USA) with a calibration range from 28 to 1380 nmol/l. T3 (nmol/l), fT4 (pmol/l) and TSH (mIU/l) were measured by an electro-chemistry-luminescence immuno-assay (ECLIA, Roche Diagnostics, Mannheim, Germany). Growth hormone was measured using a high-sensitivity chemiluminescence immunoassay with a functional assay sensitivity of 0.027 ng/ml as described recently[48]. For all measurements, levels that were not detectable were considered to have a value equal to the lower limit of detection of the assay.

In this study[15] we found that in the receiver operating characteristic curve analysis, the prognostic accuracy of cortisol was higher compared to all other measured hormones and that it was in the range of the NIHSS, similarly to copeptin. Cortisol was also an independent prognostic marker of functional outcome and death (OR 1.23 (95% CI 1.07–1.43) and 1.43 (95%CI 1.17–1.75), respectively, $p < 0.01$ for both), adjusted for age and the NIHSS, by contrast, the other anterior pituitary axis hormones i.e. TSH, peripheral thyroid hormones and GH were only of minor value to predict outcome in stroke (table 2).

Table 2. Univariate and multivariate association between hormone levels and outcome

	Univariate analysis			Multivariate analysis		
Parameter	Odds ratio	$P > z$	95% confidence interval	Odds ratio	$P > z$	95% confidence interval
Predictor: functional outcome						
Cortisol (per 100 nmol increase)	1.00	<0.0002	(1.00–1.00)	1.23	<0.01	(1.07–1.43)
fT4	1.07	0.08	(0.99–1.14)			
T3	0.32	0.01	(0.15–0.72)	0.84	0.09	(0.68–1.02)
TSH	0.78	0.01	(0.63–0.95)	0.79	0.60	(0.33–1.90)
GH	0.99	0.81	(0.90–1.08)			
Predictor: death						
Cortisol (per 100 nmol increase)	1.62	<0.0002	(1.37–1.92)	1.43	<0.001	(1.17–1.75)
fT4	1.10	0.02	(1.01–1.20)	1.08	0.13	(0.98–1.19)
T3	0.19	0.01	(0.05–0.71)	0.69	0.66	(0.13–3.60)
TSH	0.92	0.50	(0.72–1.17)			
GH	1.04	0.41	(0.95–1.14)			

Multivariate analysis was calculated for all significant predictors in univariate analysis, adjusted for age and the National Institutes of Health Stroke Scale.
fT4, free thyroxine; GH, growth hormone; T3, triiodothyronine; TSH, thyroid-stimulating hormone.

Neidert S and Katan M. et al. J Intern Med 2011[15]

Cortisol unlike copeptin (in our previous study[13]) however added no significant additional predictive value to the clinical NIHSS score. Moreover, the magnitude of association in terms of odds ratios was also less impressive if compared to copeptin.

2.2.4. Study V

Before implementing copeptin in clinical practice, we wanted to validate the prognostic potential of copeptin in a prospective, independent, large multi-center study[49]. Validation of biomarker study results is crucial, as a recent meta-analysis suggested that pilot studies on blood biomarkers typically report higher effect sizes than subsequent larger validation studies of the same blood biomarker[50].

Moreover, it was unclear whether copeptin predicts outcome in patients with ischemic stroke treated differently, that is, conservatively or with thrombolysis and if copeptin might also predict in hospital complications such as malignant cerebral edema, seizures etc. The CoRisk study aimed at validating the accuracy of copeptin in predicting functional outcome, mortality, and complications as compared to established clinical variables in a multicenter, international setting[49]. For the analysis of this study[16] we included 788 patients older than 18 years with an acute ischemic stroke within 24 hours of symptom onset, admitted consecutively to the emergency department of each tertiary care center (Berne, Basel, Frankfurt and Berlin) between March 24, 2009 and April 8, 2011. The two primary endpoints were unfavorable functional outcome (mRS 3-6 points) and mortality within 90 days. Secondary endpoint was any of the following five pre-specified complications during hospitalization: (1) symptomatic intracerebral hemorrhage according to the SITS-MOST criteria[51] (parenchymal hematoma type 2 accompanied by a four-point increase in the NIHSS score or leading to mortality), (2) space-occupying cerebral edema, (3) pneumonia (defined as auscultatory respiratory crackles combined with body temperature ≥ 38 °C, purulent sputum, or positive chest x-ray), (4) seizures (clinical diagnosis of focal and/or generalized seizure in a previously non-epileptic patient)[52] or (5) mortality within 10 days from admission. We found that higher copeptin levels again independently predicted unfavorable outcome (adjusted OR 2.17 [95% CI, 1.46–3.22], p<0.001) and mortality (adjusted OR 2.69 [95% CI, 1.64–4.41], p<0.001). Additionally we could demonstrate that copeptin was also predictive of in house

complications (adjusted OR 1.93 [95% CI, 1.33–2.80], p<0.001). The discriminatory accuracy, calculated with the area under the receiver operating characteristics curve improved significantly for all endpoints when adding copeptin to different prognostic models including the strongest clinical predictors such as the NIHSS score and age (table 3). Moreover, the combination of copeptin with a validated score encompassing both the NIHSS and age led to a net reclassification improvement of 11.8% for functional outcome and of 37.2% for mortality. Finally the predictive value of copeptin regarding functional outcome and mortality was consistent across all subgroups. We did not identify any significant effect modifiers (figure 6).

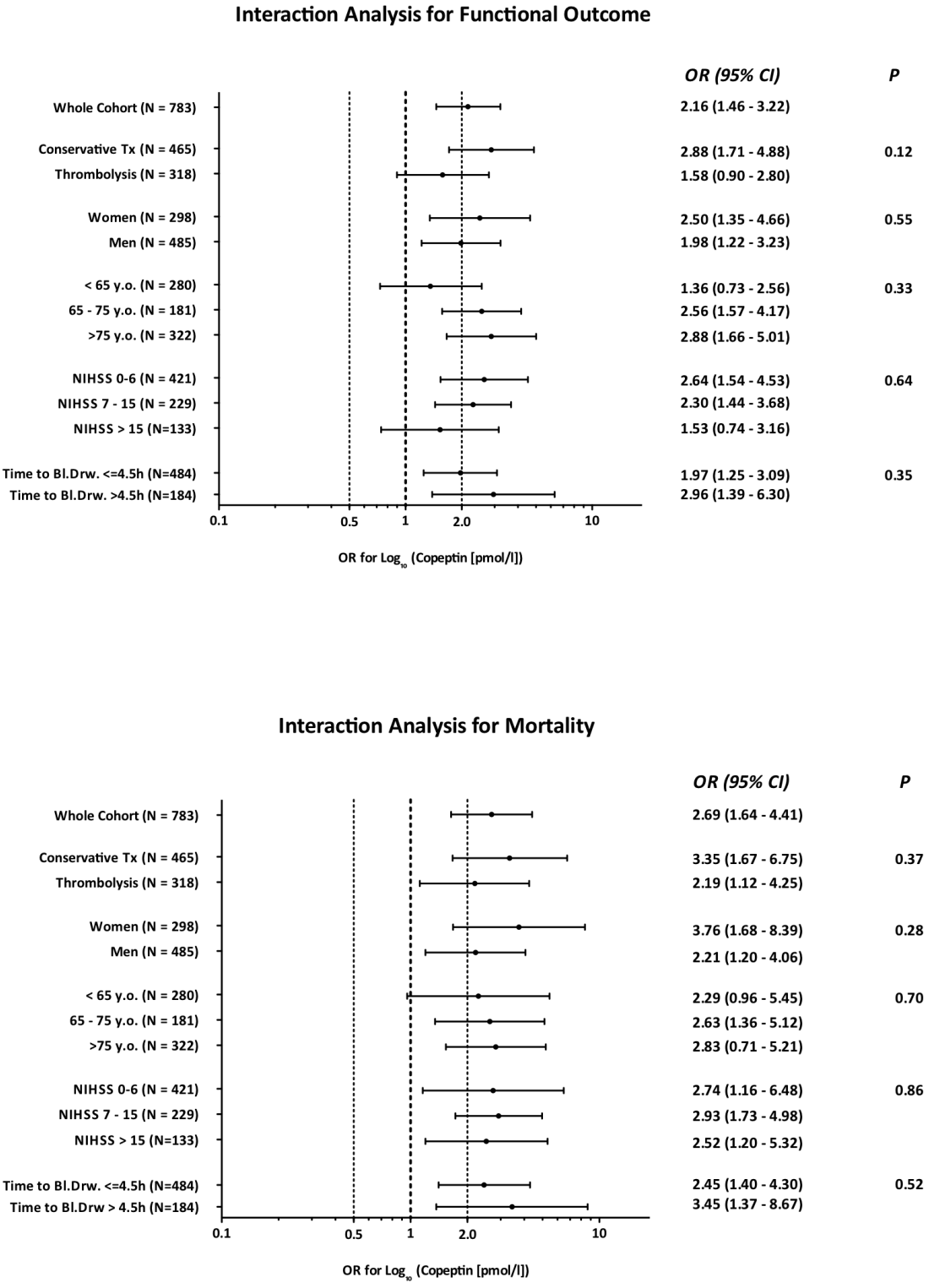
Table 3. Area under the Curve for Functional Outcome and Mortality

Functional Outcome			
Predictors	ROC Area	95% CI	P*
Copeptin [pmol/l]	0.71	0.67 – 0.75	-
NIHSS	0.81	0.78 – 0.84	<0.001
NIHSS + Copeptin [pmol/l]	0.83	0.80 – 0.86	
Model 1	0.86	0.84 – 0.89	<0.001
Model 1 + Copeptin [pmol/l]	0.87	0.85 – 0.90	
Mortality			
Predictors	ROC Area	95% CI	P*
Copeptin [pmol/l]	0.75	0.71 – 0.80	-
NIHSS	0.80	0.77 – 0.84	<0.001
NIHSS + Copeptin [pmol/l]	0.83	0.79 – 0.86	
Model 2	0.86	0.82 – 0.90	<0.001
Model 2 + Copeptin [pmol/l]	0.87	0.83 – 0.91	

*Footnote: Model 1 was the multivariate logistic regression model including all significant predictors of the univariate analysis (i.e. age, hypertension, diabetes mellitus, atrial fibrilation, modified charlson index, kidney impairment, NIHSS, TACS, Glucose, CRP, gender, DWI lesion size, unknown aetiology of stroke, time from symptom onset) and Model 2 was the cox regression model including the same predictors.*To test the statistical significane of the comparison of nested versus whole models we used the liklihood ratio test*.

DeMarchis and Katan et al., Neurology 2013[16]

Figure 6. Interaction analyses



DeMarchis and Katan et al., Neurology 2013[16]

2.3. Prediction of re-events after transient ischemic attacks

There is much interest in identifying clinical prognostic indicators that can be used to estimate stroke risk after transient ischemic attacks. The high short-term risk of stroke following TIA has been emphasized, but the ability to identify high risk patients is suboptimal[53]. Thus, comparably cheap, reliable and rapidly measureable prognostic blood markers measured in the Emergency Department may be helpful in early risk stratification and potentially improve clinical decision making in TIA patients.

2.3.1. Study VI

The present study[17] evaluated the two stress-markers copeptin and cortisol as new prognostic tools for the risk- stratification of re-events in a sub-cohort of patients with TIA derived from the aforementioned large prospective cohort (see paragraph 2.2.). The primary endpoint of this analysis was a subsequent cerebrovascular event (i.e. ischemic and hemorrhagic stroke, TIA) in the first 90 days in patients with a TIA, assessed by a structured follow up telephone interview. Stroke was defined as an acute deficit of focal neurological function with symptoms lasting more than 24 hours, resulting from intracranial vascular disturbance (ischemia or haemorrhage) occurring within 90 days after the index event. TIA was defined as an acute loss of focal cerebral or ocular function with symptoms lasting less then 24 hours of presumed ischemic origin following adequate investigations[54].

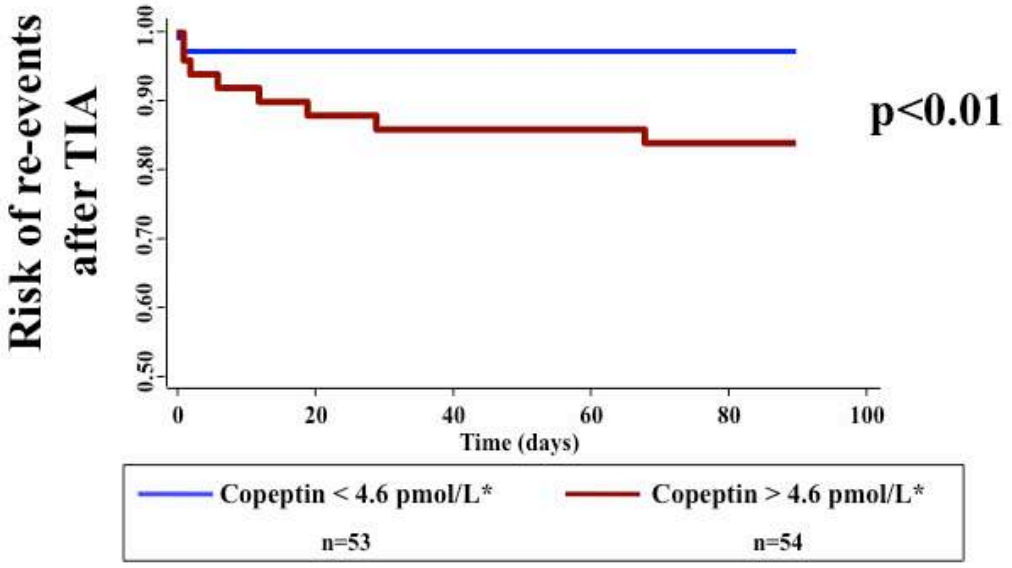
From all patients (n= 605) with a suspected cerebrovascular event, a total of 107 patients were diagnosed with a TIA on admission. All patients with acute

transient neurological dysfunction, in whom diagnostic workup suggested a non-vascular disorder, were classified as TIA mimics[54]. Risk-stratification according to the currently used ABCD2- score[55] was performed. All patients underwent a standardized diagnostic work up including specification of stroke etiology according to the TOAST classification[56], and routine laboratory testing. All plasma blood samples were obtained on admission within 72 hours of symptom onset. Copeptin was measured with a new chemiluminescence sandwich immunoassay[34] and Cortisol was measured with a competitive chemiluminescence immunoassay (IMMULITE 2000; Siemens Medical Solution Diagnostics, Los Angeles, CA, USA).

We found cerebrovascular re-events after a TIA within 90 days in ten (9%) patients, eight (8%) out of the ten patients presented with a new TIA and two (2%) had an ischemic stroke. One patient with an ischemic stroke died. Patients with a cerebrovascular re-event had higher median copeptin levels than patients without a re-event (11.25 (IQR 9.11-24.78) pmol/L) vs. 4.55 (IQR 2.8-7.8) pmol/L, p=0.016). In contrast, median basal cortisol levels in patients developing a cerebrovascular re-event compared to those without a re-event were similar (382.0 (IQR 331.0-437.5) nmol/L vs. 408 (IQR 317-557) nmol/L, p=0.53). The AUC for copeptin to predict a re-event was 0.73 (95% CI 0.545- 0.922). At a copeptin cut- off of 9.0 pmol/L, sensitivity was 80% with a specificity of 76% to diagnose a re-event. Combining copeptin and the ABCD2- score in a combined logistic regression model showed an AUC of 0.77 (95% CI 0.596- 0.949). This

combination of the clinical score and the biomarker showed a higher overall prognostic accuracy than the ABCD2- score alone (0.43 (95% CI 0.291-0.570), p= 0.002). Patients with copeptin levels below 4.60 pmol/L had a minimal risk of cerebrovascular re-events, in contrast to patients with copeptin levels above 4.60 pmol/L, who were at higher risk to develop a new cerebrovascular event (log rank p=0.02) (See Fig 7)

Figure 7. Time to re-event analysis after TIA



*Cut off level = copeptin median (to preserve power of the analysis)

Mira Katan et. al. Neurology 2011[17]

In the MRI subgroup of patients (n=88) presenting with ischemia compatible DWI lesions (n=7) (6%) copeptin levels were higher than in patients without DWI lesions (12.65 (3.75-43.55) pmol/l vs. 4.50 (2.70-7.57) pmol/l, p=0.045).

2.4. Identification of stroke etiology

At least 1 in 6 patients who survives a stroke will suffer another stroke within 5 years[1]. For optimal secondary prevention directed at the underlying mechanism, an etiological classification of stroke is critical. Even with a thorough evaluation, the etiology of ischemic stroke remains "undetermined" (i.e. classified as cryptogenic stroke according to the classical TOAST criteria[56]) or only "possible" or "probable" (according to the SSS TOAST-criteria[57]). Thus in 25-39% of patients a specific secondary prevention cannot be initiated[58]. The highest rates of stroke recurrence and mortality are seen in patients with cardioembolic and cryptogenic stroke[59]. The use of rapidly measurable serum biomarkers for etiologic diagnostic assessment on admission may enhance etiologic classification and thus improve the implementation of optimal secondary prevention, patient outcome and benefit-cost ratio.

2.4.1. Natriuretic peptides as cardioembolic markers

Interesting candidates for etiological biomarkers in acute stroke are natriuretic peptides. These peptides belong to a group of vasoactive peptide hormones whose release in part is stimulated by the sympathetic nervous system. Their physiological role is generally to maintain hemodynamics, by their natriuretic, diuretic and vasodilating actions. A-type natriuretic peptides have been proposed as biomarkers in determining atrial fibrillation and MRproANP plasma levels correlate with the duration of AF episodes[18,19]. Recently a new assay has been designed for ANP to detect the mid-region of the prohormone[60]. The mid-regional fragment of proANP is more stable than the N- or C-terminal part of the precursor

in vivo and in blood ex vivo, which renders it generally more applicable to clinical practice[61].

2.4.2. Study VII

In the earlier described stroke cohort (n=362, see also paragraph 2.2.), we thus investigated MRproANP level on admission[20]. When dividing patients into 4 subgroups based on stroke etiology, 18 % (n=65) of patients were allocated to the large artery atherosclerosis group, 36% (n=131) to the cardioembolic group, 15% (n=55) to the small vessel occlusion group, 4% (n=16) to the group of other etiologies (e.g. dissection) and 26% (n=92) to the group with cryptogenic etiology. MRproANP levels were highest in patients with CE etiology (206pmol/L (IQR 119-326), significantly higher as compared to other etiologies (124pmol/L (IQR 73-207), $p<0.0001$). In an adjusted logistic regression analysis MRproANP was independently associated with CE stroke etiology. To estimate if MR-proANP improved the diagnosis of CE etiology by already known clinical information, we calculated logistic models based on clinical information (age, known heart failure and AF on admission) and combined models based on clinical information plus MRproANP levels. The model including MRproANP (AUC 0.81 (95%CI 0.77-0.86)) was significantly better than the model based on clinical information alone (AUC 0.76 (95%CI 0.71-0.81), $p<0.001$).

2.5. Prediction of infections after stroke

Infection during the first days after ischemic stroke occurs in 25-65% of patients[62,63]. Pneumonia and urinary tract infection are the most common

infectious complications after ischemic stroke[64]. It has been suggested that the predominance of infections during the acute phase of stroke is due to stroke-induced immunosuppression[65]. The central nervous system modulates the activity of the immune system through complex pathways that include the HPA-axis, the vagus nerve, and the sympathetic nervous system[66,67]. Several studies found an independent association between stroke associated infections and poor functional outcome after ischemic stroke[68].

Therefore, early initiation of antibiotic treatments is recommended if bacterial infection is present[69]. However, gold-standard clinical diagnostics are time-consuming and delay early antibiotic therapy. Thus, accurate and readily available prognostic markers for optimal risk stratification are needed. We therefore selected C-reactive protein, white blood cells, monocytes, as they represent the most commonly measured and well-established inflammatory markers in clinical routine. Procalcitonin was selected to better discriminate bacterial infections from viral infections or general inflammation[70]. Copeptin, as reliable stress marker[33] was selected because stroke related immunosuppression may be mediated by changes in the neuroendocrine system. All these biomarkers are immediately available due to rapid analytic procedures. We hypothesize that these blood markers are predictive for the development of stroke associated infections.

2.5.1. Study VIII

In the above described stroke cohort (see paragraph 2.2.) we assessed time point and type of stroke associated infections[21] (i.e. pneumonia, urinary tract

infections, other infections). Infections were diagnosed according to the criteria of the U.S. Centre for Disease Control and Prevention[71] and stroke associated infection was defined as any infection occurring within the first 5 days of hospital admission. Time point of diagnosis was referred to the beginning of clinical symptoms, which led to diagnostic work-up and resulted in the diagnosis of infection.

In order to exclude acute infections preceding stroke, patients with admission temperature ≥ 38°C, or patients reporting an infection lasting up to 3 days before onset of stroke or patients who required mechanical intubation were not included in the study. Blood samples were collected on admission, and days 1, and 3 to assess white blood cells, monocytes, C- reactive protein, procalcitonin, and copeptin. Of 383 patients with stroke or TIA, who had at least 2 time points of measurements, 66 (17.2%) developed an infection within 5 days after onset of stroke. Twenty (5.2%) patients suffered from pneumonia, 25 (6.5%) patients had urinary tract infections and 21 (5.5%) patients had other infections (sepsis: 7 patients; phlebitis: 6 patients; gastroenteritis: 4 patients, erysipelas: 1 patient; panniculitis: 1 patient, colpitis: 2 patients). We found that copeptin, procalcitonin, white blood cells and C- reactive protein levels on admission predicted any infection, pneumonia and urinary tract infections in the acute phase of stroke in univariate analyses. After adjusting for age, NIHSS or the Charlson comorbidity index or infarct localization (infra-/supratentorial) in a bivariate model all biomarkers remained significant predictors (see table 4). The combination of the biomarkers including either white blood cells, C- reactive protein and copeptin

(AUC: 0.92) or white blood cells, C- reactive protein and procalcitonin (AUC: 0.90) showed better predictive accuracy concerning the development of pneumonia during hospitalization compared to each marker alone (p <0.0001).

Table 4. Bivariate analyses

	OR (95%CI) adjusted for age	OR (95%CI) adjusted for NIHSS	OR (95%CI) adjusted for CI	OR (95%CI) adjusted for supra−/ infratentorial infarctions
Any Infection				
Temperature	2.36 (1.48–3.75)	2.10 (1.35–3.28)	2.82 (1.46–3.56)	2.30 (1.45–3.65)
PCT	1.64 (1.27–2.12)	1.62 (1.26–2.07)	1.81 (1.37–2.40)	1.69 (1.30–2.20)
CRP	2.23 (1.72–2.90)	1.96 (1.47–2.60)	2.22 (1.70–2.90)	2.28 (1.75–2.96)
WBC	4.97 (3.42–7.21)	4.22 (2.86–6.21)	4.90 (3.34–7.20)	4.80 (3.33–6.91)
Mcyt	1.69 (1.37–2.07)	1.70 (1.37–2.10)	1.68 (1.37–2.06)	1.72 (1.40–2.11)
Copeptin	2.22 (1.64–3.02)	1.84 (1.21–2.79)	2.30 (1.72–3.70)	2.43 (1.81–3.25)
Pneumonia				
Temperature	3.11 (1.23–7.86)	2.64 (1.11–6.29)	2.95 (1.23–7.09)	2.95 (1.24–7.00)
PCT	1.89 (1.33–2.67)	1.88 (1.33–2.65)	2.15 (1.40–3.32)	1.95 (1.37–2.79)
CRP	2.58 (1.79–3.71)	2.25 (1.48–3.42)	2.60 (1.77–3.80)	2.67(1.86–3.82)
WBC	4.17 (2.41–7.22)	3.73 (2.17–6.41)	4.32 (2.58–7.23)	4.30 (2.55–7.28)
Mcyt	2.09 (1.63–2.67)	2.13 (1.65–2.75)	2.15 (1.71–2.71)	2.19 (1.72–2.79)
Copeptin	3.07 (2.08–4.53)	2.95 (1.70–5.11)	3.28 (2.24–4.81)	3.37 (2.28–4.98)
Urinary Tract Infection				
Temperature	1.66 (0.78–3.55)	1.48 (0.76–2.88)	1.61 (0.76–3.42)	1.63 (0.78–3.42)
PCT	1.56 (1.16–2.10)	1.54 (1.12–2.11)	1.74 (1.19–2.53)	1.67 (1.21–2.29)
CRP	2.21 (1.49–3.29)	1.98 (1.31–3.00)	2.21 (1.45–3.36)	2.46 (1.64–3.69)
WBC	4.50 (2.82–7.18)	4.18 (2.48–7.06)	4.76 (2.75–8.25)	4.86 (2.99–7.92)
Mcyt	1.97 (1.56–2.49)	1.99 (1.56–2.53)	2.02 (1.48–2.77)	2.08 (1.63–2.67)
Copeptin	1.86 (1.20–2.89)	1.65 (0.85–3.20)	1.92 (1.19–3.09)	2.02 (1.32–3.10)
Other Infection				
Temperature	6.94 (2.52–19.12)	5.75 (2.10–15.71)	6.57 (2.50–17.29)	6.52 (2.23–19.06)
PCT	1.29 (0.93–1.78)	1.24 (0.87–1.77)	1.37 (0.97–1.92)	1.36 0.99–1.88)
CRP	2.25 (1.50–3.37)	1.91 (1.16–3.14)	2.30 (1.53–3.44)	2.44 (1.56–3.81)
WBC	5.54 (3.49–8.78)	5.01 (2.93–8.56)	5.62 (3.48–9.08)	6.08 (3.75–9.88)
Mcyt	1.32 (0.96–1.82)	1.30 (0.95–1.79)	1.33 (0.95–1.84)	1.34 (0.95–1.91)
Copeptin	2.28 (1.36–3.79)	1.60 (0.75–3.42)	2.37 (1.50–3.74)	2.17 (1.31–3.59)

Fluri F et al. PlosONE 2012[21]

3. Discussion

The use of blood biomarkers is becoming increasingly popular in different fields of medicine. Several biomarkers are widely accepted in specific clinical situations, including troponin for the diagnosis of myocardial infarction and BNP to guide diuretic therapy in heart failure. Numerous biomarkers have also been evaluated in neurological conditions[72]. Potential clinical applications have been considered for biomarkers in vascular neurology, including: characterizing stroke size and clinical severity, identifying stroke etiology, estimating short term risk of progression or worsening, risk of vascular and overall mortality, and long term functional outcome. But at present only few studies have shown additional information on an individual basis beyond that gained from standard clinical evaluation and neuroimaging[38]. Thus none of these blood biomarkers have yet been implemented into clinical routine in the field of stroke. In order to increase successful performance of blood biomarker studies we have to comply to certain quality standards. For instance we have to use well-defined cohorts instead of case-control studies and to define a realistic and adequate number of outcome events and apply ideal statistical methods (e.g. C-statistics, goodness of fit test, net reclassification index). Furthermore, the selection process of potential candidates is crucial and should focus on a specific clinical question.

In this context my goal was to first identify and then evaluate different blood biomarkers to particularly improve risk stratification and identification of stroke etiology. In the search of new blood biomarkers for risk stratification I tried to find a marker, which is produced in the brain, which is independent of blood-brain

barrier disruption and at the same time easily accessible in the systemic circulation. The marker should as a prognostic marker ideally incorporate as much information on the body's actual condition as possible. In other words the marker should reflect to some degree the damage that has already been produced by ischemia (e.g. neuronal death), the damage that might arise (penumbra) as well as the bodies capacity to restore the damage (extend of comorbidities). Since the body has its own "alarm-system", which is mediated by the neuroendocrine-system to recognize all kinds of threats and then react and adapt, it seemed reasonable to use markers of this "alarm-system". As outlined in section 1.4, copeptin appeared to be an ideal and promising candidate and was selected for further study.

Hence, we could demonstrate for the first time in study I[12] that copeptin was a subtle marker of the individual physical stress level in a population consisting of healthy controls (no apparent physical stress), hospitalized medical patients (moderate physical stress) and surgical patients during the peri- and postoperative period (high physical stress). Specifically, copeptin showed a gradual increase with increasing levels of stress and, in contrast to cortisol levels, differentiated between healthy control subjects without apparent physical stress and medical patients with a moderate degree of stress. In addition, copeptin showed a more pronounced increase upon major stress compared to cortisol levels. The correlation between copeptin and cortisol concentrations in our study suggested that copeptin measurements are associated with the activity of the

HPA-axis. We thus concluded that copeptin is a new stress marker. Copeptin levels may reflect the stress degree at a higher, i.e. the hypothalamic-pituitary level, whereas cortisol concentrations might mirror the more peripheral stress response of the adrenal glands. If confirmed in a larger study, copeptin might provide a novel tool for the assessment of the individual stress level at the hypothalamic level.

In study II[13] we found in a large prospective stroke cohort that copeptin measured at a uniform time after stroke onset is a novel, strong, and independent prognostic marker for functional outcome and death in patients with ischemic stroke. Since no single statistical method provides all the information needed to assess a novel marker[73], we determined its discriminatory ability (using C-statistics), its accuracy (calibration by a goodness of fit test[74]) as well as the improvement of risk classification. The prognostic accuracy of copeptin in stroke patients was superior to that of other commonly measured laboratory parameters, as well as clinical measures. Importantly, copeptin is the first reported circulating biomarker that improves the prognostic accuracy of the NIHSS score significantly not only in terms of discrimination but also in terms of better risk classification. Hence copeptin if validated in an external cohort could be helpful in identifying those patients with a high risk for poor outcome, in whom more intensive neuromonitoring might be considered, as well as closer blood pressure, body temperature, and glucose adjustment. From a public health point

of view, accurate prognosis helps ensure availability of adequate resources to meet the needs of numerous stroke survivors.

In study III[14] we confirmed that copeptin is prognostic not only for short term outcome but also for long term outcome, the same magnitude of association was found and we could again demonstrate that copeptin added clinical relevant information for risk stratification according to published criteria for the evaluation of blood biomarkers in cardiovascular disease including stroke[45].

In study IV[15] we wanted to simultaneously assess hormonal markers of the anterior pituitary axis i.e. cortisol, triiodothyronine, free thyroxine, thyroid-stimulating hormone and growth hormone in patients with acute ischemic stroke with regard to their accuracy to predict functional outcome and mortality. Cortisol was found to be an independent prognostic marker of functional outcome and mortality. Conversely, triiodothyronine, free thyroxine, thyroid- stimulating hormone and growth hormone add only limited or no prognostic information to currently used measures and scores. Cortisol improved the classification of patients for functional outcome and for death in net reclassification statistics as evidenced by significant net reclassification improvements. However, the lack of significance in the combined receiver operating characteristic curve analysis suggests that these findings need to be interpreted with caution. The prognostic performance of cortisol within the same population was similar to that of

copeptin, but showed no significant additional predictive value to the NIHSS in contrast to copeptin.

Thus copeptin remained the most interesting neuroendocrine candidate as prognostic marker in the acute stroke setting. However before studying copeptin in an interventional trial to assess its value regarding patient management and ultimately cost-effectiveness we validated our results in an independent larger cohort study.

In this validation study (i.e. study V), copeptin improved the discriminatory ability of the NIHSS and multivariate models as shown by an increase in the respective AUCs. Copeptin added prognostic information to validated prognostic scores such as the index by König[75] et al, encompassing NIHSS and age. Moreover, copeptin improved reclassification compared to the multivariate models including demographic factors, cardiovascular risk factors, lesion size in MRI, comorbidities, and admission laboratory variables such as C- reactive protein and glucose. In addition, we also found that (1) copeptin independently predicted complications, and (2) that the prognostic ability of copeptin was consistent across subgroups including different acute treatments (conservative vs. thrombolysis). Study V confirmed and extended the conclusions of the previously published derivation study II. While several markers, such as NT-BNP, CRP, D-Dimers, interleukin-6, von Willebrand factor, S-100β, and neuron specific enolase, have been associated with functional outcome or mortality, only very few biomarkers contributed additional prognostic information on top of clinical

assessment[76,77]. None of these markers, however, were fully evaluated including assessment of accuracy, discriminatory ability, reclassification improvement, and performance in an external validation study according to the recommendations of the American Heart Association for studies evaluating biomarkers in cardiovascular research[45].

In study VI[17] we still considered stress markers namely cortisol and copeptin but in a different population (i.e. patients with transient ischemic attacks), having another clinical question in mind with thus another clinical endpoint (i.e. cerebrovascular re-events). As principal finding of this study, we found that copeptin, but not cortisol levels may provide additional prognostic information beyond the ABCD2-score in patients with a TIA to predict cerebrovascular re-events within 90 days.

Again copeptin performed better then cortisol for risk stratification. This was in line with our findings in study III were cortisol was not as helpful compared to copeptin concerning the prognosis in acute stroke patients or in study I, where we demonstrated that copeptin levels - even better than cortisol levels - differentiated the "no stress"- situation from "moderate stress"- situations. We hypothesized that the release of copeptin in patients suffering from a more severe “ischemic threat” (reflected also by the presence of DWI lesions in this clinically defined TIA cohort) might be more pronounced. Since this patient group is per se known to have a higher risk for cerebrovascular re-events[78] and

mortality, copeptin also seemed to be a good candidate marker for the prediction of re-events.

In study VII we assessed MRproANP besides its value as prognostic marker for functional outcome and mortality also as marker for cardioembolic stroke etiology. We hypothezised that increased MRproANP levels might mirror not only manifest heart failure but also a beginning cardiac pathology (e.g. subclinical heart failure) in which intracardial thrombus development might be more likely. This would explain the additional diagnostic value of MRproANP levels to differentiate a cardioembolic etiology from others in our study. It is essential to identify the CE etiology in stroke because recurrent stroke occurs within 2 weeks in up to 12% of patients who initially experience embolic stroke from cardiac sources[79]. Atrial fibrillation may be no longer present when patients are examined by 24-hours-electrocardiography-monitoring. Thus, anticoagulant therapy might be delayed even when the neurologist suspects an embolic origin due to distinct patterns of lesions. Using biomarkers may be a reasonable strategy to improve the identification of CE stroke already in the acute phase, as this supports the need of more extensive diagnostic tests and accelerating the start of optimal secondary prevention[80].

In study VIII we sought to identify early predictors for the development of stroke-associated infection. We evaluated routine inflammatory markers (i.e. white blood cells, monocytes, C-reactive protein) as well as procalcitonin and copeptin. The value of rapidly available blood markers as predictors of stroke associated

infections have not been studied extensively, although white blood cells CRP and monocytes are routinely measured within the first hours of admission. Procalcitonin has been evaluated in the setting of acute stroke for the prediction of stroke associated infections, however, in these studies the time point of diagnosis in relation to biomarker measurements was not taken into account. Copeptin so far was never assessed as a predictor of stroke associated infections. We found that each laboratory parameter remained a strong predictor after adjusting for NIHSS, age, Charlson comorbidity index and infarct localization. This is an unexpected finding because age and stroke severity may also contribute to stroke related immunosuppression and thus infection after acute ischemic stroke[81]. However, these biomarkers seem to add prognostic information beyond age, stroke severity and a higher Charlson comorbidity index as well as infarct localization. The predictive value of copeptin in respect to stroke associated infections was similar to that of established biomarkers of infection (i.e. WBC, CRP). This finding might be due to the association of copeptin with the activation of the HPA axis: increased copeptin-levels probably indicate a high degree of stress and stroke induced immunosuppression, which means a higher susceptibility to develop an infection. The prognostic value of procalcitonin was also in the range of white blood cells and CRP. The combination of established inflammatory makers (WBC, CRP) combined with a biomarker of stress, i.e. copeptin or a biomarker of bacterial infection, i.e. procalcitonin improves prediction of stroke-associated infections compared to the strongest prognostic marker alone. The combination of biomarkers probably

reflects better the complexity of an infection than one biomarker alone and may lead to a more accurate prediction of a beginning but not yet clinically apparent infection. The investigated biomarkers seem to detect infections before clinical or paraclincal signs prompt further diagnostic work-up leading to the diagnosis of infection. Thus these markers may help in risk stratification and may select high-risk patients for intervention studies. If validated in larger prospective studies the combination of these 3 biomarkers with best AUC values may add significant information for the early identification of high-risk patients. Future intervention studies could select patients with high-risk profiles according to these biomarker levels and these high-risk patients may proof to benefit from prophylactic antibiotic treatment.

In conclusion, there is need to develop a credible evidence base of prognostic information for outcomes that are meaningful to stroke patients and physicians to optimize care and allocation of health care resources. We discovered that with the use of neuroendocrine biomarkers, risk stratification, as well as accurate etiologic classification of stroke patients in the emergency setting, can be significantly improved. We newly identified copeptin and MRproANP as the most promising markers.

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Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level

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Abstract

BACKGROUND: During stress, vasopressin is a potent synergistic factor of CRH as a hypothalamic stimulator of the HPA axis. The measurements of CRH and vasopressin levels are cumbersome because of their instability and short half-life. Copeptin is a more stable peptide stoichiometrically released from the same precursor molecule. The aim of our study was to compare copeptin and cortisol levels in different stress situations.

METHODS: Three groups of patients with increasing stress levels were investigated: a) healthy controls without apparent stress (n=20), b) hospitalized medical patients with moderate stress (n=25) and c) surgical patients 30 minutes after extubation, with maximal stress (n=29). In all patients we assessed cortisol and copeptin levels. Copeptin levels were measured with a new sandwich immunoassay.

RESULTS: Cortisol levels in controls were (median, IQ range, 486 [397–588] nmol/L), not significantly different as compared to medical patients (438 [371–612] nmol/L, p=0.69). Cortisol levels in surgical patients after extubation were higher (744 [645–1062] nmol/L p<0.01 vs controls and medical patients). Copeptin levels in controls were 4.3 [3.2–5.5] pmol/L, which was lower as compared to medical patients (17.5 [6.4–24.1], pmol/L, p<0.001) and surgical patients after extubation (67.5 [37.8–110.0] pmol/L, p<0.001). The correlation between copeptin levels and cortisol was r=0.46, p<0.001.

CONCLUSION: Copeptin is a novel marker of the individual stress level. It more subtly mirrors different stress levels as compared to cortisol values.

ORIGINAL ARTICLE

INTRODUCTION

The two major peripheral limbs of the stress system are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system [1,2]. Corticotropin- releasing hormone (CRH) and arginin vasopressin (AVP) also termed antidiuretic hormone (ADH), are the main secretagogues of the HPA-axis to produce ACTH and cortisol, respectively. Importantly, serum cortisol levels have been reported to be proportionate to the degree of stress, if the HPA axis is intact [3,4]. For a direct assessment of the individual stress level at a cerebral level, the measurement of CRH or AVP might offer an alternative. Unfortunately, the measurement of circulating AVP levels and CRH is challenging. Both, CRH and AVP are released in a pulsatile pattern, are unstable and rapidly cleared from plasma within minutes. AVP derives from a larger precursor peptide (pre-provasopressin) along with two other peptides, neurophysin II and copeptin. Copeptin is released in an equimolar ratio to AVP and is more stable in the circulation and easy to determine. Copeptin levels were found to closely mirror the production of AVP [5].

Large surgery can serve as a standardized physiological model for studying major stress [4]. Peri- and postoperative basal cortisol levels reflect the degree of surgical stress [6,7]. Peak cortisol levels are achieved in the immediate postoperative period, around the time of extubation [3,8]. The assessment and interpretation of cortisol levels is dependent on an intact anterior pituitary and adrenal gland, respectively.

This study compares copeptin levels with the cortisol response in order to identify subtle marker of the individual stress level at a hypothalamic level.

METHODS

Setting and Study population

The details of the study population with different levels of physical stress have been previously described [4,9,10]. Briefly we obtained ethical approval and the patients or their legal representatives gave written informed consent to participate in the study. The study population was divided in to three groups; Group A (no stress) contained 20 healthy control subjects, without apparent stress. Group B (moderate stress) consisted of 25 patients consecutively recruited from a wide and representative variety of patients having been hospitalized on the medical ward. They were on the medical ward for various reasons (i.e. acute diabetic complications [hyperglycemic crisis, hypoglycemia], infections [urosepsis, exacerbation of chronic obstructive lung disease, pneumonia], inflammations [acute pancreatitis or myocarditis], hypoventilation syndrome, myocardial infarction, congestive heart failure, unstable angina pectoris, cerebrovascular insult, decompensated liver cirrhosis, and ocular neuropathy). None of the patients showed clinical or laboratory features of acute or chronic adrenal insufficiency. In Group C, 29 stable surgical patients were consecutively recruited from the division of cardiothoracic surgery undergoing elective coronary bypass grafting under general anesthesia. These patients served as a standardized model for maximal stress, which is generally experienced after extubation [3,8]. For premedication midazolam 7.5mg was used in all patients. Anesthesia was induced with thiopentone (2–4mg/kg) and fentanyl (2–4ug/kg). Etomidate was not used for any patient. Intubation was facilitated with pancuronium (0.15 mg/kg). Before and during cardiopulmonary bypass anesthesia was maintained with isoflurane (0.5–1.5 MAC) and fentanyl. Antibiotic prophylaxis consisted of cefuroxime (1,5g, t.i.d.) for 48h. Surgery was done under normothermic conditions (i.e. resulting in cooling not lower than 35° Celsius). Cardiopulmonary bypass was started after heparin (350 IU/kg) and cylocapron 30 mg/kg. No blood transfusions were required. None of the patient's received corticosteroid treatment.

Exclusion criteria were as follows: patients who received drugs that influence the hypothalamo-pituitary adrenal axis in the last 3 months [11]; patients with diseases affecting the adrenal or the pituitary gland; patients with known or suspected primary or secondary adrenal insufficiency; and patients receiving etomidate.

In Group A and Group B, patients were tested once between 6–9 a.m. Surgical patients in Group C were evaluated at three different time points - the morning (6-9 a.m.) before operation (no apparent stress) and 30 minutes after extubation (with maximal stress) as well as on the day after the operation.

Adrenal function in each participant was assessed by either low or standard ACTH tests performed between 0600–0900 h. Thereby, in all subjects, blood samples were taken at 0 min for the basal measurement of cortisol and ACTH and again at 30 and 60 min for the measurement of serum cortisol concentration after iv administration of 1 or 250 μg Synacthen (synthetic ACTH1–24), respectively.

Assay

We measured Copeptin serum levels with a new sandwich immunoassay as recently described in detail [12]. Briefly, this sandwich immunoluminometric assay works with two polyclonal antibodies which bind to the C-terminal region of pre-pro-AVP. One antibody is attached to polystyrene tubes and the other is labeled with acridinium ester for chemiluminescence detection. For the assay 50 μl of either serum or plasma are required, no extraction steps or other pre-analytical procedures like addition of protease inhibitors are necessary. The analytical detection limit is 1.7 pmol/L and the total precision of the assay (inter laboratory CV) was < 20% for copeptin concentrations across the calibration curve (up to 405 pmol/L). In 359 healthy individuals Copeptin plasma concentration had a median of 4.2 pmol/L (range, 1–13.8 pmol/L). The 97.5th percen-

Table 1: Characteristics of the study group (Group A-C)

	Healthy controls (Group A, n=20)	Medical patients (Group B, n=25)	Surgical patients undergoing CABG (Group C, n=29)	
			preoperative	postoperative
Age (yr)	51(35–57)	64(56–79)	67(59–75)	
Sex (m/f)	10/10	15/10	28/1	
BMI (kg/m2)	23(21–24)	29.7(25–31)	27(24–28)	
Mean blood pressure (mmHg)		89(86–97)	90(83–93)	85(77–90)
Sodium (mmol/l)		141(138–144)	141(139–143)	138(136–138)
Potassium (mmol/l)		4.0(3.6–4.3)	4.0(3.7–4.3)	4.3(4.2–4.7)
Glucose (mmol/l)		5.8(5.3–7.8)	5.9(5.3–7.7)	7.8(7–9.1)

Demographic and laboratory characteristics of healthy controls (group A), medical patients (group B), and surgical patients undergoing coronary artery bypass grafting (CABG) (group C) before the operation and after surgery. To convert glucose from mmol/liter into mg/dl, multiply by 18. m, male; f, female. BMI denotes body mass index.

tile was 11.25 pmol/L, and the 2.5th percentile was 1.7 pmol/L. Of all 359 tested volunteers, only nine (2.5%) had a plasma copeptin concentration below the detection limit of the assay of 1.7 pmol/L. It is important to note that in contrast to mature AVP, copeptin is more stable in plasma or serum ex vivo. Ex vivo stability of copeptin (< 20% loss of recovery) was shown for serum and plasma for at least seven days at room temperature and 14 days at 4° C[12].

Statistical Analysis

We expressed discrete variables as counts (percentage) and continuous variables as means ± standard deviation (SD) or median [interquartile range] unless stated otherwise. Two-group comparison of normally distributed data was performed by Students t-test. For multigroup comparisons, one-way analysis of variance with least square difference for posthoc comparison was applied. For data not normally distributed, we performed the Mann-Whitney-U test. The Kruskal-Wallis one-way analysis of variance was used if more than two groups were being compared. Correlation analyses were performed by using Spearman rank correlation. All testing was two-tailed and P values less than 0.05 were considered to indicate statistical significance.

RESULTS

Demographic data

Table 1 shows demographic and other characteristics of the subjects in Group A-C.

We investigated 20 healthy controls, 25 medical patients and 29 patients undergoing surgical treatment. Twenty-one subjects (28.4%) were female. In the surgical group, all except one patient were male. There was no significant difference between the three groups concerning age, body mass index, blood pressure, and electrolytes.

Cortisol levels in different stress situations

Cortisol levels in controls were 486 [397–588] nmol/L not significantly different as compared to medical patients (basal cortisol 438 [371–612], p=0.69). Cortisol levels in surgical patients 30 minutes after extubation were significantly higher (744 [645–1062], p<0.01) as compared to controls and medical patients **(Figure 1A)**.

Copeptin levels in different stress situations

Copeptin levels in controls were 4.3 [3.2–5.5] pmol/L, which was significantly lower as compared to medical patients (17.5 [6.4–24.1], p<0.001) and surgical patients after extubation (67.5 [37.8–110.0] pmol/L, p<0.001) **(Figure 1B)**.

Percentage increase and decrease of cortisol and copeptin levels in different stress situations in surgical patients (Group C)

The percentage increase in cortisol from baseline to the extubation period (i.e. major stress) was 265 ± 34%, and the respective increase for copeptin was significantly higher with 1430 ± 157% (p<0.001) **(Figure 2).**

Cortisol levels the day after the operation decreased to 82.3 ± 46.2% compared to the immediate extubation period, defined as maximal stress (p=0.01), whereas copeptin levels decreased to 65.5% ± 34.1 (p<0.001). The decrease for copeptin levels tended to be more pronounced as compared to the decrease in cortisol levels (p=0.09).

Copeptin levels and gender

In control subjects, copeptin levels were significantly lower in females (4.56± 2.36 pmol/L) compared to copeptin levels in males (6.16 ± 2.30; p=0.025) .In the group of medical patients copeptin levels were not significantly different between men and women.

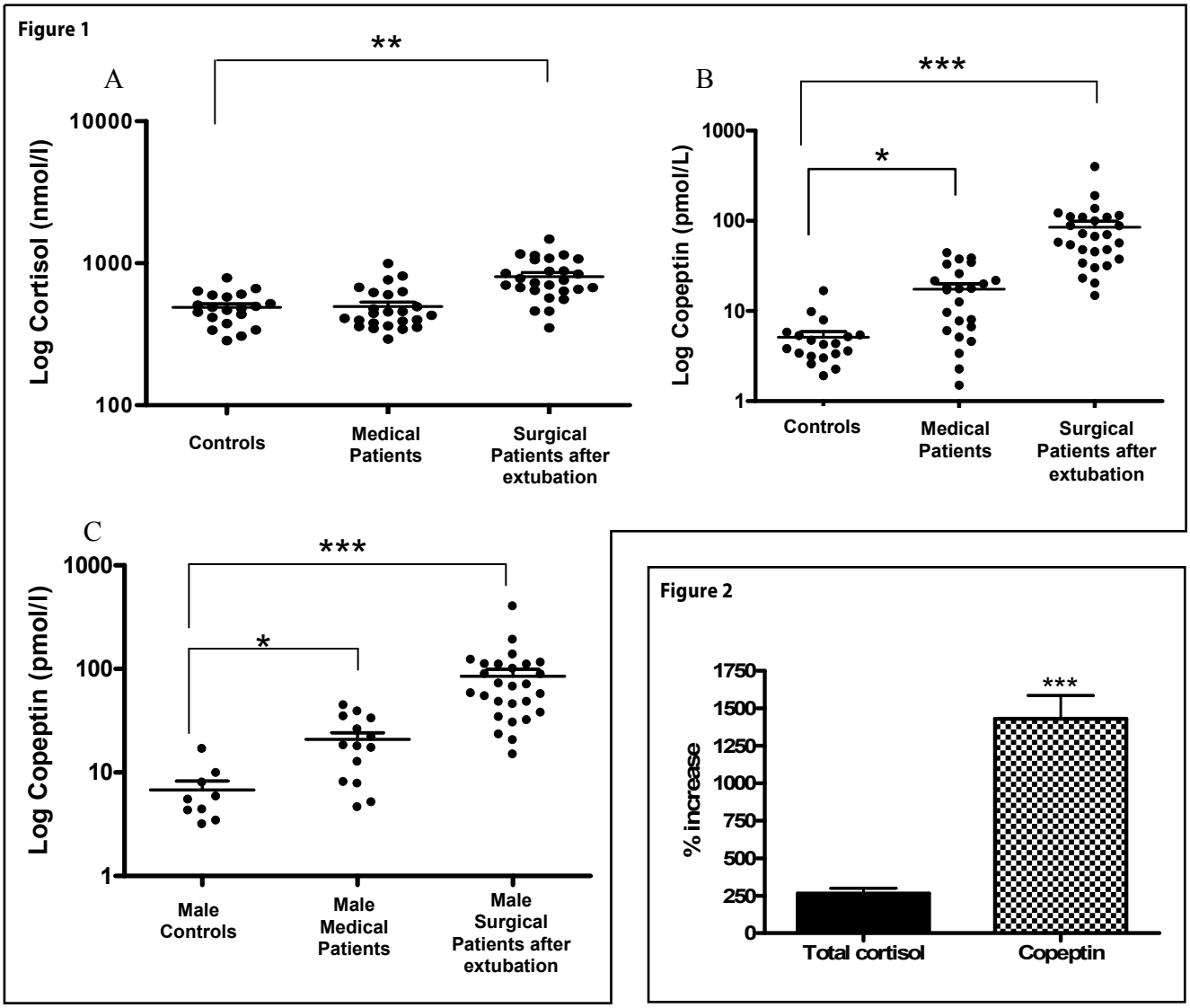

Figure 1. Total cortisol (A) and copeptin (B) levels in controls, medical patients and surgical patients after extubation, mirroring three different levels of physical stress. Copeptin levels (C) in the male controls, male medical patients and male surgical patients. Data show means ± SEM with scatter plots representing the absolute values. * denotes p<0.05; ** denotes p<0.01; *** denotes p<0.001

Figure 2. (A)Percentage increase of total cortisol and copeptin levels from before operation (no apparent stress) to after extubation (major stress). (B) Percentage decrease of total cortisol and copeptin levels from after extubation (major stress) to the day after operation.
Data present mean ± SEM. * denotes p<0.05; ** denotes p<0.01; *** denotes p<0.001

The study results remained similar when performing the analyses only in the male subpopulation **(Figure 1C).**

The correlation between copeptin levels and cortisol was r=0.46, $p<0.001$ There was no correlation between copeptin levels and age or between copeptin and sodium values. No patient had dysnatremia.

DISCUSSION

In our cohort, copeptin was found to be a more subtle marker of the individual stress level. Specifically, copeptin shows a gradual increase with increasing levels of stress and, in contrast to cortisol levels, differentiates between healthy control subjects without apparent stress and medical patients with a moderate degree of stress. In addition, copeptin shows a more pronounced increase upon major stress as compared to cortisol levels.

The significant correlation between copeptin and cortisol concentrations in our study indicates that both

measurements mirror the activity of the HPA-axis. Copeptin levels reflect the stress degree at a higher, i.e. the hypothalamic-pituitary level, whereas cortisol concentrations mirror the more peripheral stress response of the adrenals.

Presently, in clinical routine, cortisol levels are considered to predict the ability of our body to produce an adequate stress response [10]. However, cortisol levels only mirror the peripheral endocrine response of the adrenals to stress, whereas the hypothalamic response to stress is not detected. CRH and AVP might play a crucial role in conducting the perception of stress at the central hypothalamic level. There is an emerging view that AVP is the principal regulated variable that imparts situation specific drive on the HPA axis, whereas CRH serves mainly to impose stimulatory tone [13]. Since AVP measurement is problematic, measurement of copeptin in this regard provides a novel and valuable laboratory tool.

Our study is observational in nature. Thus, we can not make a conclusion about the clinical impact of the higher increase in copeptin levels compared to serum cortisol values upon major stress. Possibly, copeptin provides a more direct and earlier mirror of the stress level as compared to cortisol. Our finding that copeptin levels, but not cortisol levels, increase in medical patients with a moderate stress level compared to healthy subjects without stress strengthens this assumption.

AVP levels mirror fluid balance. Since copeptin is stoichiometrically produced with AVP, one could argue that the increase of copeptin levels may be explained by a change in water balance. However, plasma sodium as main parameter of osmolality was not different between medical and surgical patients at the time-point where we measured copeptin. This suggests that changes in osmolality were not the main stimulus for the increase in copeptin. As a limitation, we did not assess serum osmolality or detailed fluid balance in our patients.

We found significantly lower baseline copeptin levels in female versus male control subjects which is in accordance with the published literature. Morgenthaler et al. found also that copeptin values in healthy individuals differed significantly between men and women [12]. In contrast, we found no significant difference in the copeptin levels upon increasing stress level between men and women. In addition, analyzing only male study subjects showed a similar gradual increase of copeptin upon increasing levels of stress compared to the whole study population. Thus, in situations of more severe stress, the baseline gender differences are overridden by the increasing stress response.

Another well known stimulus of AVP secretion is a drop in blood volume and, thus, blood pressure. This might have influenced the pronounced increase of copeptin in patients after extubation and is part of a severe stress response of the body. However a small reduction in blood pressure usually has little or no effect on plasma vasopressin levels [14]. In our study population blood pressure between medical patients and surgical patients was not significantly different; thus, a drop in blood pressure can not explain the increase of copeptin levels in medical patients, again suggesting that the increase in copeptin levels was predominantly stress-related.

Copeptin has already been evaluated in critically ill patients with septic shock [15–17], in patients with respiratory infections [18,19] as well as upon changes in osmolality [20] and in patients with diabetes insipidus [21]. In the acute state, copeptin, similarly to cortisol, has been shown to be an independent marker for reduced survival in critically ill patients as well as in patients with heart insufficiency [22]. Measurement of plasma copeptin concentrations in critically ill patients deserves to be evaluated as an indirect laboratory parameter to assess stress and therefore outcome in future studies.

Our study has limitations. First, this is a preplanned posthoc analysis of an observational study evaluating cortisol levels in different stress situations and the diagnostic performance of low dose and standard ACTH tests. Our findings should, therefore, be regarded as hypothesis-generating. Second, our study population included patients with intact adrenal function, as assessed by the ACTH-test and can therefore only yield information about the acute stress level in patients with preserved adrenal function. Therefore, we can not draw conclusions about the impact of adrenal insufficiency on copeptin levels. For example patients with complete or relative adrenal insufficiency might provoke a hypersensitivity to a stressor resulting in higher basal levels of copeptin and possibly predicting a worse outcome. We further did not perform serial blood sampling although cortisol levels show large variations during the day. However, during critical illness and severe stress, the circadian pattern of cortisol is usually lost [23]. Nevertheless, serial cortisol measurements would most probably have given more reliable results.

In conclusion, copeptin subtly mirrors the individual stress level in a population consisting of healthy controls, hospitalized medical patients and surgical patients during the peri- and postoperative period. It shows a more gradual increase with increasing stress as compared to total cortisol levels. If confirmed in a larger subset of patients, copeptin might provide a novel tool for the assessment of the individual stress level at the hypothalamic level.

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Copeptin: A Novel, Independent Prognostic Marker in Patients with Ischemic Stroke

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Objective: Early prediction of outcome in patients with ischemic stroke is important. Vasopressin is a stress hormone. Its production rate is mirrored in circulating levels of copeptin, a fragment of provasopressin. We evaluated the prognostic value of copeptin in acute stroke patients.

Methods: In a prospective observational study, copeptin was measured using a new sandwich immunoassay on admission in plasma of 362 consecutive patients with an acute ischemic stroke. The prognostic value of copeptin to predict the functional outcome (defined as a modified Rankin Scale score of ≤ 2 or ≥ 3), mortality within 90 days, was compared with the National Institutes of Health Stroke Scale score and with other known outcome predictors.

Results: Patients with an unfavorable outcomes and nonsurvivors had significantly increased copeptin levels on admission ($p < 0.0001$ and $p < 0.0001$). Receiver operating characteristics to predict functional outcome and mortality demonstrated areas under the curve of copeptin of 0.73 (95% confidence interval [CI], 0.67–0.78) and 0.82 (95% CI, 0.76–0.89), which was comparable with the National Institutes of Health Stroke Scale score but superior to C-reactive protein and glucose ($p < 0.01$). In multivariate logistic regression analysis, copeptin was an independent predictor of functional outcome and mortality, and improved the prognostic accuracy of the National Institutes of Health Stroke Scale to predict functional outcome (combined areas under the curve, 0.79; 95% CI, 0.74–0.84; $p < 0.01$) and mortality (combined areas under the curve, 0.89; 95% CI, 0.84–0.94; $p < 0.01$).

Interpretation: Copeptin is a novel, independent prognostic marker improving currently used risk stratification of stroke patients.

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Acute ischemic stroke is a devastating disease; it is the third leading cause of death and the leading cause of acquired disability in developed countries.[1] In 2008, an estimated 780,000 persons in the United States will suffer from a stroke, and 15 to 30% of stroke survivors will be permanently disabled. The direct and indirect cost of stroke is expected to amount to approximately $65.5 billion.[2] An early risk assessment with estimate of the severity of disease and prognosis is pivotal for optimized care and allocation of healthcare resources.[3] A prompt identification of patients at increased risk for adverse outcome interventions could be targeted to those most likely to benefit. In this context, rapidly measurable markers predicting mortality and functional outcome in stroke would be clinically helpful.

Abnormalities in endocrine function have been reported in ischemic stroke with activation of the hypothalamo-pituitary-adrenal axis being one of the first measurable physiological responses to cerebral ischemia.[4–6] Vasopressin (AVP) is a potent synergistic factor of corticotropin-releasing hormone as hypothalamic stimulator of the hypothalamo-pituitary-adrenal axis. Small studies found increased AVP levels in patients with ischemic stroke,[7] correlating with stroke severity.[8] However, the measurement of AVP levels is cumbersome because of its instability and short half-life. AVP derives from a larger precursor peptide (provasopressin) together with two other peptides, neurophysin II and copeptin. Copeptin is released in an equimolar ratio to AVP, and is more stable in the circulation and easy to measure.[9] This study aimed at prospectively evaluating the prognostic value of copep-

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tin levels in a large cohort of patients with an acute ischemic stroke and comparing it with other known outcome predictors.

Subjects and Methods

Study Design and Setting

We conducted a prospective cohort study at the University Hospital Basel, Basel, Switzerland. From November 2006 to November 2007, all patients with an acute ischemic cerebrovascular event were included. The Ethics Committee of Basel, Switzerland, approved the trial protocol. Informed consent was obtained from patients before enrolment; otherwise, the consent was obtained from the patients' next of kin or a physician not involved in the study, in case next of kin were absent. This report adheres to the consolidated standards for the reporting of observational trials.[10]

Patients

Patients were eligible for inclusion if they were admitted to the emergency department with an acute ischemic stroke defined according to the World Health Organization criteria[11] and with symptom onset within 72 hours.

Clinical Variables

On admission (ie, during the first 24 hours), the following items were recorded: vital signs; relevant comorbidities as assessed by the Charlson comorbidity index (CCI) adjusted for stroke (the CCI is a comorbidity scoring system that includes weighting factors by disease severity according to the ICD-9-CM system)[12]; medication before stroke; thrombolysis (intravenous/intraarterial) as acute stroke treatment; risk factors (ie, age; sex; smoking history; hypercholesterolemia; history of hypertension, diabetes mellitus, previous ischemic stroke, or transient ischemic attack, respectively; positive family history for myocardial infarction, stroke, or transient ischemic attack); and severity of stroke as assessed by the National Institutes of Health Stroke Scale (NIHSS) score[13] (scores range from 0 to 42, with greater scores indicating increasing severity), performed by a stroke neurologist certified in the use of this scale. The clinical stroke syndrome was determined applying the criteria of the Oxfordshire Community Stroke Project, that is, total anterior circulation syndrome (TACS), partial anterior circulation syndrome, lacunar syndrome, and posterior circulation syndrome.[14] Patients underwent the following standardized diagnostic workup to exclude intracranial hemorrhage and to identify the cause of cerebral infarction: brain computer tomography, magnetic resonance imaging (MRI), or both; standard 12-lead electrocardiography; 24-hour electrocardiography; echocardiography; and neurosonographic studies of the extracranial and intracranial arteries. Routine laboratory testing was always done. Stroke cause was determined according to the criteria of the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification,[15] which distinguishes large-artery atherosclerosis, cardioembolism, small-artery occlusion, other causative factor, and undetermined causative factor.

Blood Sampling and Follow-up

Blood samples were collected on admission (within 0–3 [n = 78], 3–12 [n = 189], 12–24 [n = 55], and 24–72 [n = 40] hours from symptom onset). In patients who died within 24 hours after admission or in patients who were discharged, data from admission or until discharge were collected. The NIHSS score was assessed on admission and day 5. Functional outcome was obtained on days 5 and 90 according to the modified Rankin Scale (mRS)[16] blinded to copeptin levels.

End Points

The primary end point of this study was favorable functional outcome of stroke patients after 90 days from baseline, defined as an mRS score of 0 to 2 points. Secondary end point in stroke patients was death from any cause within a 90-day follow-up. Outcome assessment was performed by two trained medical students blinded to copeptin levels with a structured follow-up telephone interview with the patient or, if not possible, with the closest relative or family physician.

Neuroimaging

CCT was performed in all patients on admission mainly to exclude intracranial hemorrhage. Thereafter, MRI was performed on a clinical 1.5-Tesla MR Avanto system (Siemens, Erlangen, Germany) using a stroke protocol, including T1-, T2-, and diffusion-weighted imaging (DWI) sequences, and a magnetic resonance angiography. MRI with DWI was available in 197 stroke patients (55%). In those patients, DWI lesion volumes were determined by consensus of two experienced raters unaware of the clinical and laboratory results. The lesion size was calculated by the commonly used semiquantitative method.[17] Lesions were ranked into three sizes to represent typical stroke patterns: (1) small lesion with a volume of less than 10ml, (2) medium lesion of 10 to 100ml, and (3) large lesion with a volume of more than 100ml.[18]

Assays

Blood was obtained from an indwelling venous catheter. Results of the routine blood analyses were recorded. Plasma was frozen at −70°C. Measurement was done in a single batch with a commercial sandwich immunoluminometric assay (B.R.A.H.M.S LUMItest CT-proAVP, B.R.A.H.M.S AG, Hennigsdorf/Berlin, Germany), as described in detail elsewhere.[19] Since this initial publication, the assay was modified as follows: The capture antibody was replaced by a murine monoclonal antibody directed to amino acids 137 to 144 (GPAGAL) of provasopressin. This modification improved the sensitivity of the assay. The lower detection limit was 0.4pmol/L, and the functional assay sensitivity (<20% interassay coefficient of variance) was less than 1pmol/L. Median copeptin levels in 200 healthy individuals was 3.7pmol/L, and the 97.5 percentile was 16.4pmol/L. The median in healthy individuals using this modification was similar as published in other studies (4.2pmol/L in Morgenthaler and colleagues[19] and 3.8pmol/L in Khan and colleagues[20]).

Statistical Analysis

Discrete variables are summarized as counts (percentage), and continuous variables as medians and interquartile ranges (IQRs). Two-group comparison of not normally distributed data was performed using Mann–Whitney *U* test, and a Kruskal–Wallis one-way analysis of variance was used for multigroup comparisons.

To investigate whether copeptin allows predicting of both functional outcome and death after 3 months, we used different statistical methods. First, the relation of copeptin with the two end points was investigated with the use of logistic regression models. Therefore, common logarithmic transformation (ie, base 10) was performed to obtain normal distribution for skewed variables (ie, copeptin concentrations) as the resulting model yielded smaller Akaike Information Criterion, which was chosen to compare the results. We used crude models and multivariate models adjusted for all significant outcome predictors and report odds ratios (ORs). Note that the OR corresponds to a one-unit increase in the explanatory variable; for the log-transformed copeptin values, this corresponds to a 10-fold increase.

Second, we compared different prognostic risk scores from different predictive models by calculating receiver operating characteristic analysis. Thereby the area under the receiver operating characteristic curve (AUC) is a summary measure over criteria and cut-point choices. The AUC summary equals the probability that the underlying classifier will score a randomly drawn positive sample higher than a randomly drawn negative sample. To test whether the copeptin level improves score performance, we considered the two nested logistic regression models with NIHSS and copeptin as compared with NIHSS only. Under the lower-dimensional submodel, the difference in deviance between the two models has a χ^2 distribution with 1 degree of freedom. Furthermore, care was taken to adjust for the optimistic bias of in-sample prediction error estimates using a fivefold cross-validation scheme. Letting Y be the indicator of the event of interest and X the covariate vector of a given risk score, high utility corresponds to accurately modeling the regression $E(Y \mid X = x)$. We used the Brier's score as the quadratic scoring rule to measure predictive performance, where the fitted values of the predictive probabilities $\Pr(Y = 1 \mid X = x)$ are contrasted with the actually observed values.

Third, to assess the calibration of the predictive models, we compared the number of events that are observed with those that are expected by estimation from the models within different a priori risk groups based on Goldstein and colleagues'[21,22] data.

Fourth, we calculated reclassification tables to further investigate the benefit of copeptin level as compared with the NIHSS score alone on risk as proposed by, for example, Cook[23] and Pencina and coauthors.[24] Regarding so-called net reclassification improvement, only those changes in estimated prediction probabilities that imply a change from one risk category to another were considered. For estimating meaningful a priori risk categories, we used predicted probabilities based on Goldstein and colleagues'[21,22] data.

Finally, to study the ability of copeptin for mortality prediction, we calculated Kaplan–Meier survival curves and stratified patients by copeptin tertiles.

All testing was two tailed, and p values less than 0.05 were considered to indicate statistical significance. All calculations were performed using STATA 9.2 (Stata Corp, College Station, TX), R version 2.8.1 and the ROCR package (version 1.0–2), which is available from CRAN repository (http://cran.r-project.org/), for evaluating and visualizing the performance of scoring classifiers.[25]

Results

Patients

From 605 screened patients, ischemic stroke was diagnosed in 362 patients, and 359 completed follow-up and were included in the analysis (Fig 1). Furthermore, we excluded 11 patients from the analysis because of missing values for copeptin.

Descriptive Characteristics of Stroke Patients

The median age of patients with ischemic stroke included in this study was 75 (IQR, 63–83) years and 41% were women. On admission, the median NIHSS score was 5 (IQR, 2–10) points. Median body temperature was 37.0°C (IQR, 36.5–37.4°C), and the median systolic blood pressure was 160mm Hg (IQR, 140–180mm Hg). A total of 275 patients (77%) had a history of hypertension, 93 (26%) had hypercholesterol-

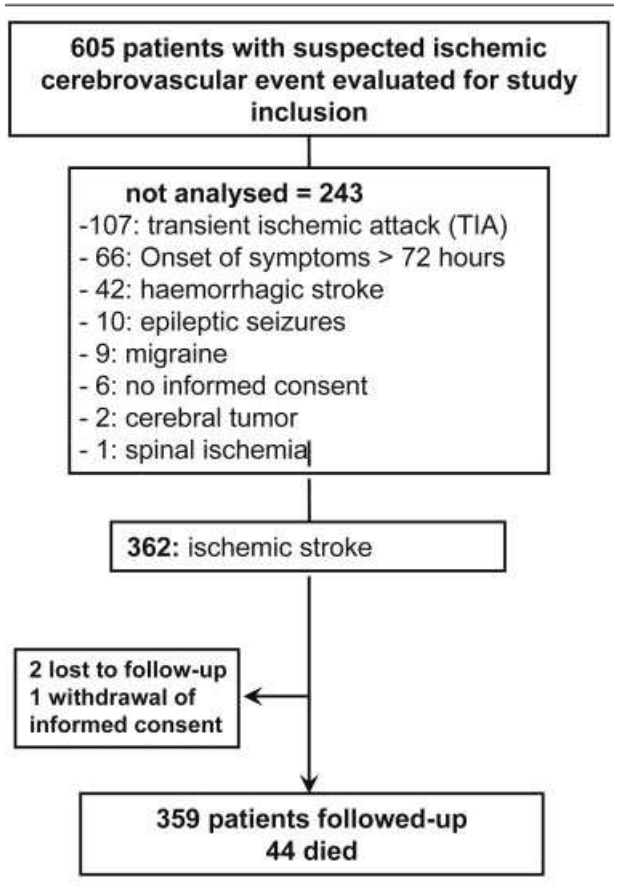

Fig 1. Study profile/flow sheet of the study.

emia, 71 (20%) had a history of diabetes mellitus, 124 (35%) were smokers, 75 (21%) were diagnosed with atrial fibrillation, 88 (25%) had a history of a previous vascular event, and 91 (25%) had coronary heart disease. An unfavorable functional outcome was found in 151 patients (42%) with a median mRS score of 4 (IQR, 3–6). Forty-four patients died, and the mortality rate was thus 12%. The principal baseline characteristics of all patients grouped according to their functional outcome are provided in Table 1.

Main Results

COPEPTIN AND SEVERITY OF STROKE ACCORDING TO THE NATIONAL INSTITUTES OF HEALTH STROKE SCALE AND LESION SIZE. Copeptin levels increased with increasing severity of stroke as defined by the NIHSS score. Copeptin concentrations in patients with an NIHSS score of 0 to 6 points (n = 210) were 8.6 (IQR, 5.2–15.3) pmol/L, in patients with an NIHSS score of 7 to 15 points (n = 86) were 15.8 (IQR, 7.7–28.7) pmol/L, and in patients with an NIHSS score greater than 15 points (n = 52) were 30.1 (IQR, 9.0–67.9) pmol/L. In the subgroup of patients (n = 197) in whom MRI was available, copeptin levels paralleled lesion size. Median copeptin levels in patients with a small lesion were about half the levels than in patients with medium lesions (8.4 [IQR, 4.4–13.7] vs 14.9 [IQR, 6.6–26.0] pmol/L), whereas levels were greatest in patients with a large lesion (18.3 [IQR, 5.3–51.9] pmol/L).

COPEPTIN AND FUNCTIONAL OUTCOME AFTER 3 MONTHS. Copeptin levels in patients with an unfavorable outcome were significantly greater than those in patients with a favorable outcome (19.4 [IQR, 8.7–36.6] vs 8.2 [IQR, 4.5–14.5] pmol/L; $p < 0.0001$; Fig 2A).

In univariate logistic regression analysis, we calculated the ORs of log-transformed copeptin levels as compared with the NIHSS score and other risk factors as presented in Table 2. With an unadjusted OR of 6.9 (95% CI, 3.89–12.33), copeptin had a strong association with functional outcome. After adjusting for all other significant outcome predictors, copeptin remained an independent outcome predictor with an adjusted OR of 2.53 (95% CI, 1.26–5.07). In addition, age, CCI, presence of TACS, and the NIHSS score remained significant outcome predictors, unlike all others assessed (Table 3). In the subgroup of patients (n = 197) in whom MRI evaluations were performed, copeptin was an independent outcome predictor with an OR of 2.89 (95% CI, 1.14–7.34; $p = 0.026$) after adjustment for both lesion size (OR, 1.01; 95% CI, 1.00–1.02; $p = 0.043$) and the NIHSS score (OR, 1.07; 95% CI, 1.01–1.14; $p = 0.027$).

With an AUC of 0.73 (95% CI, 0.67–0.78), copeptin showed a significantly greater discriminatory ability as compared with CCI, sex, and the presence of TACS, and was within the range of the NIHSS score and age (Table 4). In addition, copeptin was superior to C-reactive protein (CRP) (AUC, 0.61; 95% CI, 0.55–0.68; $p < 0.001$), white blood cell count (AUC, 0.55; 95% CI, 0.49–0.62; $p < 0.0001$), and glucose (AUC, 0.57; 95% CI, 0.50–0.63; $p < 0.001$). Copeptin improved the NIHSS score (AUC of the combined model, 0.79; 95% CI, 0.74–0.84; $p < 0.001$). This improvement was stable in an internal 5-fold cross-validation that resulted in an average AUC (standard error) of 0.748 (0.036) for the NIHSS and 0.789 (0.028) for the combined model, corresponding to a difference of 0.041 (0.014). The 5-fold cross-validated mean squared prediction error decreased from 0.197 (0.012) in the model with NIHSS to 0.181 (0.012) in the model with NIHSS and copeptin, corresponding to an average decrease of 0.016 (0.002). Moreover, a model combining copeptin level, age, sex, and the CCI with the NIHSS score showed an AUC of 0.85 (95% CI, 0.81–0.89), which was greater than all predictors alone ($p < 0.0001$) (see Table 4).

COPEPTIN AND DEATH WITHIN 90 DAYS. Copeptin levels in 41 of the 44 patients who died were more than 3 times greater as compared with patients who survived (9.5 [IQR, 5.3–19.1] vs 35.6 [IQR, 19.4–93.7] pmol/L; $p < 0.001$; see Fig 2B). Univariate analysis identified copeptin levels, age, presence of TACS, and the NIHSS score as the main predictors associated with death (see Table 2). After adjustment for these parameters, copeptin level remained an independent predictor for mortality with an OR of 4.23 (95% CI, 1.605–11.152; see Table 3).

Receiver operating characteristic curves demonstrated the greatest discriminatory accuracies for copeptin level (AUC, 0.83; 95% CI, 0.76–0.89) and the NIHSS score (AUC, 0.85; 95% CI, 0.78–0.91). The combination of copeptin level and the NIHSS score had a higher discriminatory accuracy (AUC, 0.89; 95% CI, 0.84–0.94) than the NIHSS score alone ($p = 0.02$). In addition, the combination of age, copeptin, TACS, and the NIHSS score showed the greatest accuracy (AUC, 0.92; 95% CI, 0.88–0.95), greater than all individual parameters alone ($p < 0.01$) (see Table 4).

Again, this improvement was stable in an internal 5-fold cross-validation with an AUC (standard error) of 0.837 (0.018) for the NIHSS and 0.886 (0.019) for the combined model. Thus, the average difference across cross-validation runs was 0.049 (0.020). Improvement in the fitted values of the predictive probabilities for 5-fold cross-validated Brier's score was 0.071 (0.009) for the logistic model with copeptin as com-

Table 1. **Baseline Characteristics of Stroke Patients**

Baseline Characteristics	All	Good Outcome (mRS 0–2)	Poor Outcome (mRS 3–6)	p[a]
n	359	208	151	
Demographic data				
Median age, yr (IQR)	75 (63–83)	71 (59–80)	80 (71–86)	<0.001
Female sex (n)	41% (149)	35% (73)	49% (76)	<0.01
Stroke severity, median NIHSS score (IQR)	5 (2–10)	4 (2–6)	8 (4–17)	<0.001
DWI lesion size	197	139	58	
Small	136	74% (103)	56% (33)	<0.001
Medium	50	24% (33)	30% (17)	<0.001
Big	11	2% (3)	14% (8)	<0.001
Laboratory findings	359	208	151	
Median copeptin level, pmol/L (IQR)[b]	11.6 (5.6–21.2)	8.2 (4.5–14.5)	19.4 (8.7–36.6)	<0.0001
Median glucose level, mmol/L (IQR)	6.1 (5.5–7.4)	6.0 (5.3–7.2)	6.3 (5.6–7.7)	<0.05
Median C–reactive protein concentration, mmol/L (IQR)	3.6 (3.0–9.9)	3.0 (3.0–6.5)	4.90 (3.0–19.9)	<0.001
Median white blood cell count (IQR)	8.2 (6.5–9.9)	8.1 (6.5–9.6)	8.3 (6.6–10.1)	NS
Vital parameters on admission	359	208	151	
Median arterial pressure, mm Hg (IQR)				
Systolic	160 (140–180)	162 (143–180)	159 (132–180)	NS
Diastolic	90 (80–100)	91 (81–102)	90 (79–98)	NS
Median body temperature, °C (IQR)	37.0 (36.5–37.4)	37.0 (36.7–37.5)	37 (36.4–37.4)	NS
Stroke causative factors[c]	359	208	151	
Small-vessel occlusive (n)	15% (55)	18% (38)	11% (17)	NS
Large-vessel occlusive (n)	18% (65)	18% (38)	18% (27)	NS
Cardioembolic (n)	36% (131)	36% (75)	37% (56)	NS
Other (n)	4% (16)	5% (11)	3% (5)	NS
Unknown (n)	26% (92)	22% (46)	30% (46)	NS
Therapies before admission	359	208	151	
Antihypertensive (n)	59% (213)	57% (119)	62% (94)	NS
ASS (n)	37% (133)	36% (74)	39% (59)	NS
Clopidogrel (n)	5% (18)	3% (7)	7% (11)	NS
Anticoagulant (n)	11% (38)	9% (18)	13% (20)	NS
Statins (n)	22% (78)	23% (47)	21% (31)	NS
Comorbidity	359	208	151	
Median Charlson Index (IQR)	1 (0–2)	0 (0–2)	1 (0–2)	<0.0001
Vascular risk factors	359	208	151	
Hypertension (n)	77% (275)	73% (152)	81% (123)	<0.05
Atrial fibrillation (n)	21% (75)	16% (34)	27% (41)	<0.05
Smoking history (n)	35% (124)	38% (79)	30% (45)	NS
Hypercholesterolemia (n)	26% (93)	28% (58)	23% (35)	NS
Diabetes mellitus (n)	20% (71)	19% (39)	21% (32)	NS
Coronary heart disease (n)	25% (91)	23% (48)	28% (43)	NS
Prior stroke (n)	25% (88)	23% (48)	26% (40)	NS
Family history for stroke and/or myocardial infarction (n)	30% (106)	32% (67)	26% (39)	NS
Stroke syndrome	359	208	151	
TACS (n)	11% (41)	5% (11)	20% (30)	<0.0001
PACS (n)	45% (162)	44% (92)	47% (69)	NS
LACS (n)	21% (74)	23% (47)	18% (27)	NS
POCS (n)	23% (83)	28% (58)	16% (24)	<0.01

[a]p value was assessed using Mann-Whitney U test.
[b]In 11 patients, values for copeptin were missing.
[c]Some had two causative agents at the same time and because of rounding, percentages may not sum to 1.
mRS = modified Rankin Scale; IQR = interquartile range; NIHSS = National Institutes of Health Stroke Scale; DWI = diffusion-weighted imaging; NS = not significant; TACS = total anterior circulation syndrome; PACS = partial anterior circulation syndrome; LACS = lacunar syndrome; POCS = posterior circulation syndrome.

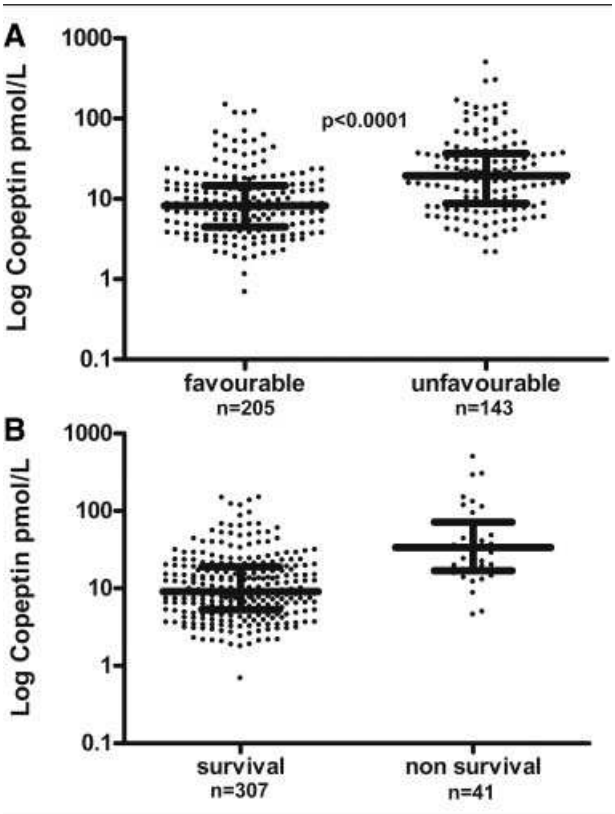

Fig 2. (A) Copeptin levels in stroke patients with favorable and unfavorable functional outcome. (B) Copeptin levels in survivors and nonsurvivors of stroke. Mann–Whitney U *Test. All data are medians and interquartile ranges (IQR), with* dot plots *representing all values.*

pared with 0.076 (0.013) for NIHSS only. This corresponds to an average decrease of 0.005 (0.005).

The time to death was analyzed by Kaplan–Meier survival curves based on copeptin tertiles. Patients in the lowest tertile (copeptin <7.2pmol/L) had a minimal risk for death, in contrast with patients with copeptin levels in the 2nd and 3rd tertiles (copeptin between 7.2 and 17.8pmol/L and copeptin > 17.8pmol/L, respectively) (p <0.0001) (Fig 3).

Reclassification

In-sample reclassification behavior for patients with good functional outcome and patients with poor functional outcome (see Supplementary Table 5A) and for patients who died and for those who did not die (see Supplementary Table 5B) was calculated (see supplementary material). Forty patients with poor outcome were classified in higher risk categories using the model with the NIHSS and copeptin. Twenty patients with poor outcome were classified in lower risk categories using the model with the NIHSS and copeptin as compared with the model with the NIHSS score as the only predictor variable. Thus, the estimated net reclassification improvement for functional outcome was 0.3935. Similarly, 10 patients who died were classified in higher risk categories and 2 patients who died were classified in lower risk categories using the model with the NIHSS score and copeptin level as compared with the model with the NIHSS score as the only predictor variable. The estimated net reclassification improvement for mortality was 0.4818.

Discussion

In this prospective, observational study, we found that copeptin is a novel, strong, and independent prognostic marker for functional outcome and death in patients with ischemic stroke. The prognostic accuracy of copeptin in stroke patients is superior to that of other commonly measured laboratory parameters, as well as clinical measures. It is in the range of the commonly used clinical NIHSS score. Importantly, copeptin is the first reported circulating biomarker that improves the prognostic accuracy of the NIHSS score significantly.

The NIHSS is a standardized measure of stroke severity and is used to predict 3-month outcome. However, it has some limitations. The use of the NIHSS implies special training and there remains a notable interobserver variability.[26] In addition, left hemispheric stroke syndromes show greater NIHSS scores than right hemispheric syndromes, and the NIHSS is less reliable in patients with posterior compared with anterior circulation syndromes.[27] Early and adequate risk assessment is pivotal for optimized care of stroke patients. In this context, readily measurable biomarkers, such as copeptin, are additionally helpful in predicting the severity level and outcome of patients with ischemic stroke.

When recorded on admission, different variables are allegedly associated with poor outcome in stroke patients, for example, body temperature,[28] blood glucose,[29] CRP,[30] and white blood cell count.[31] Compared with control subjects, CRP levels were greater in patients with stroke than in healthy control subjects in all stroke subtypes, both in the acute phase and after a 3-month follow-up.[32] It has been demonstrated that high-sensitive CRP was an accurate prognostic marker for mortality.[33] However, other studies found no differences in CRP concentrations in a mixed stroke population,[34] and pretreatment CRP failed to predict outcome in stroke patients treated with intravenous thrombolysis.[35] All of these parameters were modest predictors of functional outcome and unable to improve the prognostic accuracy of the NIHSS.

Other mediators involved in the ischemic cascade are protein S-100β,[36] interleukin-6,[37] matrixmetalloproteinase-9,[38] myelin basic protein, and neuron-specific enolase.[39] Although these biomarkers reliably mirror the initial stroke severity (NIHSS and lesion size) and some even bear an association with outcome, they were not able to independently predict functional

Table 2. **Univariate Analysis**

Predictors	Functional Outcome			Mortality		
	OR[a]	95% CI[a]	*p*	OR	95% CI[a]	*p*
Copeptin (increase per log unit)[b]	6.93	3.89–12.33	<0.0001	16.11	6.95–37.35	<0.0001
CRP (increase per unit)	1.01	1.00–1.02	0.01	1.10	0.99–1.21	0.05
Glucose (increase per unit)	1.07	0.97–1.18	0.15	1.01	1.00–1.02	**0.009**
Age (increase per unit)	1.06	1.04–1.08	**<0.0001**	1.09	1.05–1.13	**<0.0001**
Temperature (increase per unit)	0.87	0.61–1.24	0.44	0.86	0.50–1.48	0.59
Systolic blood pressure (increase per unit)	0.99	0.99–1.01	0.45	0.99	0.98–1.00	0.15
Female sex	1.78	1.17–2.74	0.01	1.35	0.72–2.54	0.93
NIHSS (increase per unit)	1.16	1.12–1.21	**<0.0001**	1.19	1.14–1.25	**<0.0001**
Risk factors						
Charlson Index (increase per unit)	1.34	1.15–1.56	**<0.0001**	1.16	0.96–1.40	0.13
Hypertension	1.62	1.00–2.70	0.06	1.75	0.75–4.09	0.19
Atrial fibrillation	1.91	1.14–3.19	0.01	3.16	0.92–6.15	0.09
Smoking history	0.69	0.44–1.08	0.11	0.61	0.29–1.24	0.16
Hypercholesterolemia	0.78	0.48–1.27	0.32	0.96	0.47–1.97	0.90
Diabetes mellitus	1.17	0.70–2.00	0.57	1.06	0.48–2.31	0.89
Coronary heart disease	1.33	0.82–2.14	0.25	2.07	1.07–4.00	0.03
Prior stroke	1.20	0.75–1.95	0.46	0.66	0.29–1.48	0.31
Family history for stroke and/or MI	0.74	0.46–1.17	0.19	0.89	0.44–1.80	0.756
Stroke syndrome and causative factors						
TACS	4.44	2.15–9.19	**<0.0001**	6.67	3.39–13.99	**<0.0001**
PACS	1.12	0.74–1.69	0.74	0.81	0.41–1.54	0.53
LACS	0.75	0.44–1.26	0.28	0.35	0.12–1.02	0.054
POCS	0.489	0.287–0.831	0.01	0.500	0.200–1.231	0.202
Small-vessel occlusive	0.58	0.30–1.05	0.07	0.113	0.015–0.843	0.033
Large-vessel occlusive	0.97	0.56–1.68	0.93	0.55	0.21–1.45	0.23
Cardioembolic	1.05	0.68–1.62	0.84	1.54	0.82–2.92	0.43
Other	0.61	0.21–1.80	0.37	0.47	0.06–3.64	0.46
Unknown	1.54	0.96–2.49	0.08	1.96	1.02–3.79	0.05

[a]Note that the odds ratio corresponds to a unit increase in the explanatory variable; for copeptin, this corresponds to an increase per unit of the log transformation of copeptin (thus, a log-transformed increase of 1 corresponds to a copeptin increase of 10pmol/L).
[b]In 11 patients, values for copeptin were missing.
OR = odds ratio; CI = confidence interval; CRP = C-reactive protein; NIHSS = National Institutes of Health Stroke Scale; MI = myocardial infarction; TACS = total anterior circulation syndrome; PACS = partial anterior circulation syndrome; LACS = lacunar syndrome; POCS = posterior circulation syndrome.

outcome or death within 3 months or to improve the predictive value of the NIHSS, in the earlier mentioned studies. It has been demonstrated that brain natriuretic peptide and its N-terminal peptide (NT-proBNP) are excellent markers for vascular mortality and re-events.[40] However, baseline levels of NT proBNP were not significantly associated with functional outcome within 3 months.[41]

AVP, together with corticotropin-releasing hormone, is the main secretagogue of the hypothalamo-pituitary-adrenal axis to produce adrenocorticotropic hormone and cortisol. Serum cortisol levels have been reported to increase proportionately with the degree of stress and to predict outcome in several diseases[42] including ischemic stroke[43]; however, it has not been shown that cortisol is able to improve the prognostic accuracy of the NIHSS. We have recently shown that copeptin levels mirror different levels of stress more subtly than cortisol.[44] In addition, cortisol shows cross-reactivity with other steroids,[45] varies with the amount of hormone-binding proteins, underlies a circadian rhythm,[46] and changes with food intake,[47] thus limiting its prognostic accuracy in the acute phase of stroke.

Copeptin is known to have prognostic value in non-neurological diseases. For example, copeptin levels are independent predictors of survival in critically ill patients suffering from hemorrhagic and septic shock.[48] Furthermore, copeptin levels have prognostic implications in patients with acute heart failure[49] and in patients with acute myocardial infarction.[20]

We assume that the close and reproducible relation of copeptin levels to the degree of activation of the

Table 3. **Multivariate Analysis**

Predictor	OR	95% CI[a]	*p*
Multivariate Analysis for Functional Outcome			
Copeptin (increase per log unit)	2.57	1.27–5.17	0.01
Age (increase per unit)	1.06	1.04–1.09	**<0.0001**
Female sex	1.43	0.82–2.49	0.21
Stroke severity, NIHSS (increase per unit)	1.17	1.10–1.23	**<0.0001**
Charlson Index (increase per unit)	1.31	1.09–1.58	**0.004**
TACS	1.51	0.55–4.15	0.42
Multivariate Analysis for Mortality			
Copeptin (increase per log unit)	4.31	1.65–11.25	**0.003**
Age (increase per unit)	1.07	1.03–1.12	**0.002**
Stroke severity, NIHSS (increase per unit)	1.16	1.09–1.23	**<0.0001**
TACS	1.52	0.51–4.55	0.458

OR = odds ratio; CI = confidence interval; NIHSS = National Institutes of Health Stroke Scale; TACS = total anterior circulation syndrome.

Table 4. **Prediction of Functional Outcome and Mortality**

Parameter	AUC	95% CI		*p*
Prediction of Functional Outcome				
Copeptin	0.73	0.67	0.78	
NIHSS	0.75	0.70	0.80	0.46
Age	0.70	0.64	0.76	0.47
Charlson Index	0.63	0.58	0.69	**<0.01**
Sex	0.58	0.52	0.63	**<0.001**
TACS	0.57	0.54	0.61	**<0.0001**
Combined score (NIHSS/copeptin)	0.79	0.75	0.84	**<0.01**
Prediction of Mortality				
Copeptin	0.82	0.76	0.89	
NIHSS	0.85	0.78	0.91	0.59
Age	0.74	0.67	0.81	0.08
TACS	0.64	0.57	0.72	**<0.001**
Combined score (NIHSS/copeptin)	0.89	0.84	0.94	**0.01**

AUC = area under the curve; CI = confidence interval; NIHSS = National Institutes of Health Stroke Scale; TACS = total anterior circulation syndrome.

stress axis, and thus disease severity, is the basis of its unique usefulness as a biomarker. Seemingly, copeptin allows tapping an endogenous information system of our body at a hypothalamic level that, through mechanisms still poorly understood, accurately assesses the severity of damage. Copeptin mirrors somehow the individual "stress burden" of patients. If the individual threshold, that is, to ensure homeostasis, is crossed, it is most likely that a less favorable outcome occurs.

The following limitations of our study must be taken into account. First, we analyzed patients within a rather large time frame of 72 hours of symptom onset, representing a heterogeneous stroke population. We intended to reflect a general, unselected population as it occurs in clinical routine, and results remained similar when analyzing only patients with symptom onset within 0 to 12, 12 to 24, and 24 to 72 hours. Furthermore, in the subgroup of patients who underwent thrombolysis (symptom onset 0–3 hours; n = 78), copeptin levels measured on admission were greater in

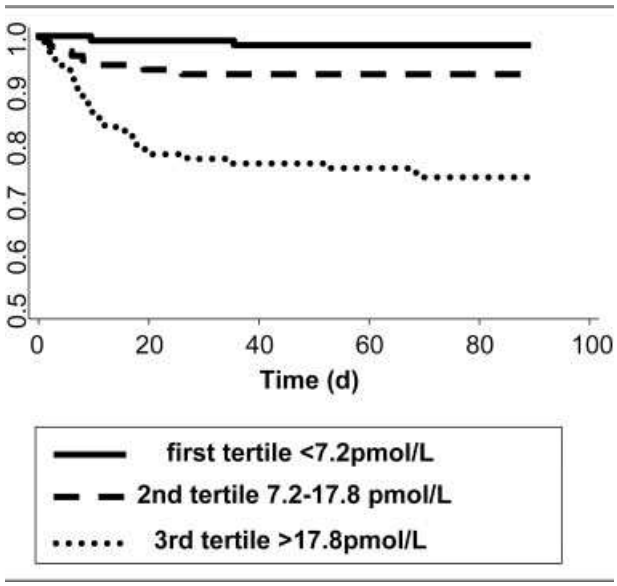

Fig 3. Kaplan–Meier survival curves for copeptin. Solid line *represents the first tertile (<7.2pmol/L);* dashed line *represents second tertile (7.2–17.8pmol/L);* dotted line *represents the third tertile (>17.8pmol/L).*

patients with unfavorable outcome compared with patients with favorable outcome, and the prognostic accuracy for functional outcome and mortality was similar to the overall sample. Second, we performed DWI in only a subset of patients. However, this mirrors clinical routine where MRI testing is not yet widely available within this time window in the emergency setting.

Despite its inherent limitations, outcome predictors are helpful in identifying those patients with a high risk for poor outcome, in whom more intensive neuromonitoring might be considered, as well as closer blood pressure, body temperature, and glucose adjustment.

Moreover, it is important to develop a credible evidence base of prognostic information for outcomes that are meaningful to patients and relatives, including level of independency. From a public health point of view, accurate prognosis helps ensure availability of adequate resources to meet the needs of numerous stroke survivors.

It is customary to base the prognostic assessment and treatment decisions on several parameters that each mirror different pathophysiological aspects. In this context, copeptin appears to have an interesting potential as a new prognostic biomarker and makes it a promising candidate also for a multimarker panel. This may allow improved risk stratification and allocation of targeted therapies for stroke patients in the future. However, before broad implementation, additional studies are needed for external validation.

Disclosure

N.G.M. is employee of B.R.A.H.M.S., the manufacturer of the copeptin-assay (B.R.A.H.M.S CT-proAVP LIA, B.R.A.H.M.S AG, Hennigsdorf/Berlin, Germany). B.M. has served as consultant and received payments from B.R.A.H.M.S., to attend meetings, for speaking engagements, and for research unrelated to this trial.

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Prognostic Value of Copeptin : One-Year Outcome in Patients With Acute Stroke

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Prognostic Value of Copeptin
One-Year Outcome in Patients With Acute Stroke

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Background and Purpose—An accurate long-term outcome prediction may improve management of stroke patients. We investigated the ability of copeptin to predict 1-year outcome in stroke patients.

Methods—In this preplanned post hoc analysis, the National Institutes of Health Stroke Scale score and copeptin levels were measured on admission in a cohort of patients with ischemic stroke. The primary end point was functional outcome (modified Rankin Scale score <3 or 3–6) after 1 year. The secondary end point was all-cause mortality.

Results—Of 362 patients, 341 (94.2%) completed the 1-year follow-up, 146 (43%) patients had an unfavorable functional outcome, and 66 (20%) died. Multivariate logistic-regression analysis adjusted for age and National Institutes of Health Stroke Scale score showed that copeptin was an independent predictor of functional outcome (odds ratio=4.00; 95% CI, 1.94–8.19) and death (odds ratio=2.68; 95% CI, 1.24–5.82). The area under the receiver operating characteristic curve of copeptin was 0.72 (95% CI, 0.67–0.77) for functional outcome and 0.74 (95% CI, 0.69–0.78) for mortality. Copeptin improved the area under the receiver operating characteristic curve of the National Institutes of Health Stroke Scale score for functional outcome from 0.70 (95% CI, 0.64–0.74) to 0.76 (95% CI, 0.71–0.82; $P=0.002$) and for mortality from 0.74 (95% CI, 0.69–0.78) to 0.78 (95% CI, 0.71–0.85; $P=0.04$).

Conclusions—Copeptin levels are a useful, complementary tool to predict functional outcome and mortality 1 year after stroke.

Clinical Trial Registration—ISCTRN 00390962; clinicaltrials.gov No. NCT00390962.

(*Stroke*. 2010;41:1564-1567.)

Key Words: biomarkers ■ copeptin ■ 1-year outcome ■ stroke

Stroke ranks second to ischemic heart disease as a cause of death worldwide and is the leading cause of serious disability.[1] Mortality after 1 year ranges between 21% and 27%; 15% to 30% of survivors are permanently disabled.[1] Costs of long-term care account for ≈50% of total costs.[2] Early treatment and adequate rehabilitation are known to reduce dependency at 6 months and may improve long-term outcome.[2] Prediction of long-term outcome at stroke onset based on clinical deficits only is difficult[3]; therefore, rapid measurement of blood biomarkers predicting long-term functional outcome and mortality could prove useful. We recently demonstrated in a prospective cohort study that the biomarker copeptin predicted outcome 90 days after stroke onset and improved the prognostic accuracy of the National Institutes of Health Stroke Scale (NIHSS) score.[4] This follow-up study evaluated copeptin as a marker to predict functional outcome and mortality in acute stroke patients 1 year after admission.

Patients and Methods

Setting

This study was based on a prospective cohort study (ClinicalTrials.gov number, NCT00390962).[4] A complete description has been reported previously.[4] In brief, vital signs, relevant comorbidities, risk factors, and stroke severity on admission as assessed by the NIHSS were recorded. Copeptin was measured with a sandwich immunoluminometric assay.[5] For follow-up, we used structured telephone interviews performed by 1 trained medical student, blinded to copeptin levels.[6] The primary end point of this study was functional outcome in patients 1 year after stroke, defined by a modified Rankin Scale score (unfavorable outcome=modified Rankin Scale score >2). The secondary end point was death from any cause within the 1-year follow-up.

Statistical Analysis

Discrete variables are expressed as counts (percentages) and continuous variables as medians and interquartile ranges (IQRs). Two-group and multigroup comparisons were performed with the

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Table. Univariate and Multivariate Analyses

	One-Year Functional Outcome				One-Year Mortality			
Predictor	Univariate Odds Ratio (95% CI)*	*P*	Multivariate Odds Ratio (95% CI)*	*P*	Univariate Odds Ratio (95% CI)*	*P*	Multivariate Odds Ratio (95% CI)*	*P*
Copeptin†	7.1 (3.9–12.8)	<0.0001	4.0 (1.9–8.2)	<0.0001	7.8 (4.0–15.3)	<0.0001	2.7 (1.2–5.8)	0.01
CRP	1.0 (0.9–1.0)	0.32			1.0 (1.0–1.0)	0.08		
Age	1.1 (1.1–1.1)	<0.0001	1.1 (1.0–1.1)	<0.0001	1.1 (1.1–1.2)	<0.0001	1.1 (1.1–1.1)	<0.0001
Systolic blood pressure	1.0 (1.0–1.0)	0.27			1.0 (1.0–1.0)	0.47		
Female sex	1.7 (1.1–2.6)	<0.05	1.2 (0.7–2.1)	0.49	1.9 (1.1–3.3)	<0.05		
Stroke severity, NIHSS score	1.1 (1.1–1.2)	<0.0001	1.1 (1.1–1.2)	<0.0001	1.1 (1.1–1.2)	<0.0001	1.1 (1.1–1.2)	<0.0001
Risk factors								
Hypertension	1.3 (0.8–2.2)	0.31			1.5 (0.8–3.1)	0.21		
Atrial fibrillation	2.1 (1.2–3.5)	<0.01	0.7 (0.3–1.4)	0.29	3.4 (1.9–6.1)	<0.0001	1.3 (0.6–2.7)	0.56
Smoking history	0.6 (0.4–1.0)	<0.05	1.0 (0.6–1.8)	0.93	0.4 (0.2–0.8)	<0.01	0.8 (0.4–1.8)	0.63
Hypercholesterolemia	0.7 (0.4–1.1)	0.16			0.7 (0.4–1.4)	0.34		
Diabetes mellitus	1.4 (0.8–2.3)	0.24			0.9 (0.5–1.8)	0.85		
Coronary heart disease	1.2 (0.8–2.0)	0.39			1.2 (0.7–2.2)	0.56		
Prior stroke	1.2 (0.7–2.0)	0.42			1.0 (0.5–1.8)	0.91		
Family history of stroke/ myocardial infarction	0.5 (0.3–0.8)	<0.01	0.6 (0.3–1.1)	0.11	0.6 (0.4–1.2)	0.17		
Stroke syndrome								
TACS	3.3 (1.6–6.8)	<0.01	1.3 (0.5–3.6)	0.65	3.3 (1.7–6.8)	<0.01	0.9 (0.3–2.7)	0.87
PACS	1.0 (0.6–1.5)	0.98			1.5 (0.9–2.5)	0.15		
LACS	0.8 (0.5–1.4)	0.42			0.4 (0.2–0.9)	<0.05		
POCS	0.6 (0.4–1.1)	0.08			0.5 (0.2–1.0)	<0.05		
Stroke etiology								
Small-vessel occlusive	0.6 (0.3–1.1)	0.09			0.2 (0.1–0.7)	<0.05		
Large-vessel occlusive	0.8 (0.5–1.5)	0.55			0.5 (0.2–1.1)	0.07		
Cardioembolic	1.0 (0.6–1.5)	0.89			1.4 (0.8–2.4)	0.25		
Other	0.9 (0.3–2.5)	0.77			0.6 (0.1–2.8)	0.52		
Unknown	1.8 (1.1–2.9)	<0.05	1.5 (0.8–2.8)	0.20	2.3 (1.3–4.1)	<0.01		

TACS indicates total anterior circulation syndrome; PACS, partial anterior circulation syndrome; LACS, lacunar circulation syndrome; and POCS, posterior circulation syndrome.

*Note that the odds ratio corresponds to a unit increase in the explanatory variable; †for copeptin, this corresponds to an increase per unit of the log transformation of copeptin (thus, a log-transformed increase of 1 corresponds to a copeptin increase of 10 pmol/L).

Mann–Whitney *U* test and Kruskal-Wallis 1-way ANOVA, respectively. The relation of copeptin to outcomes was assessed in logistic-regression models. For multivariate analysis, we included confounders, known risk factors, and other outcome predictors as assessed in univariate analysis. Receiver-operating-characteristic curves were calculated to assess discrimination. To estimate the additive benefit of copeptin to the NIHSS score, we used likelihood ratio tests. Kaplan–Meier survival curves were constructed for mortality prediction. All testing was 2 tailed, and probability values <0.05 were considered statistically significant. Calculations were performed with STATA 9.2 (Stata Corp, College Station, Tex) and Graphpad Prism 5 (Graphpad Software).

Results

Patients

Of the original 362 ischemic stroke patients,[4] 341 (94.2%) completed the 1-year follow-up. In 5 patients, copeptin values were missing, leaving 336 patients for the final analysis. Baseline characteristics have been reported previously.[4] The median age of the patients was 75 (IQR, 65 to 83) years, 40% were women, and the median NIHSS score on admission was 5 points (IQR, 2 to 10).

Primary End Point

In the 146 patients (43%) with an unfavorable functional outcome, copeptin levels were higher compared with those in patients with a favorable outcome (19.30; IQR, 9.60 to 36.98 pmol/L vs 8.12; IQR, 4.58 to 14.65 pmol/L; $P<0.0001$). Univariate and multivariate logistic-regression analyses showed that copeptin was associated with functional outcome (Table). The area under the curve (AUC) of copeptin was 0.72 (95% CI, 0.67 to 0.77) and significantly higher than for white blood cell count (WBC), C-reactive protein (CRP), and glucose but similar to the AUC for NIHSS score (0.70; 95% CI, 0.64 to 0.76).

We stratified patients into copeptin tertiles and compared outcome within 6 predefined NIHSS risk categories.[7] Increasing copeptin tertiles were associated with

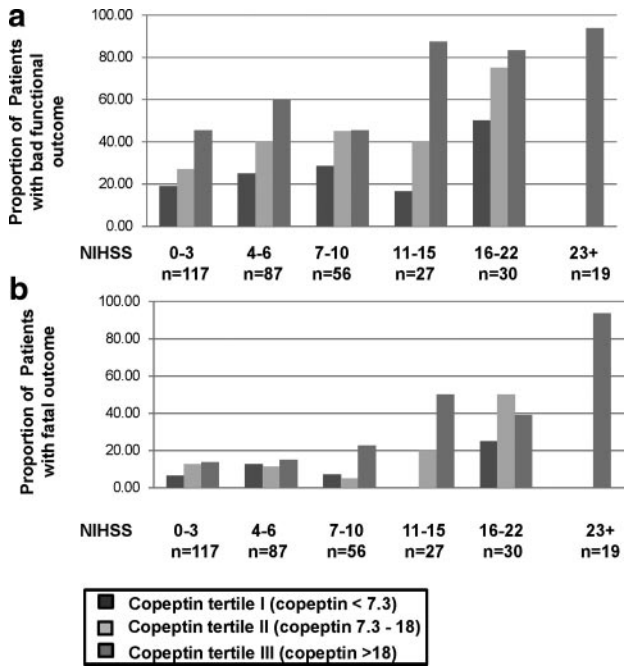

Figure 1. Bar graph of NIHSS score in combination with copeptin for (a) functional outcome and (b) mortality prediction.

higher risk for adverse outcome (Figure 1a). Similarly, the combination of copeptin and NIHSS score in a combined logistic-regression model improved the NIHSS score (AUC of the combined model=0.78; 95% CI, 0.72 to 0.84; $P<0.01$). This was not true for glucose, CRP, or WBC.

Secondary End Point

Sixty-six patients (20%) died. Copeptin levels in nonsurvivors (28.10 pmol/L; IQR, 13.20 to 60.88) were higher compared with those in survivors (9.34 pmol/L, IQR, 5.37 to 19.00; $P<0.0001$). In univariate and multivariate analyses, copeptin was an independent predictor for mortality. Receiver-operating-characteristic analysis showed similar results for the NIHSS score (AUC=0.74; 95% CI, 0.66 to 0.81) and copeptin (AUC=0.74; 95% CI, 0.69 to 0.78) but inferior results for WBC, CRP, and glucose. Stratification of patients into copeptin tertiles confirmed increased mortality risk in 6 predefined NIHSS risk groups (Figure 1b). The combination of NIHSS score and copeptin improved the AUC to 0.79 (95% CI, 0.71 to 0.87; $P<0.05$). We found an increased risk for mortality with increasing copeptin tertiles, particularly from the second to the third tertile (Figure 2).

Discussion

The ability to use biochemical markers to improve the prognostic accuracy after acute ischemic stroke is attractive.[8] Several biomarkers were evaluated previously: brain natriuretic peptide, CRP, WBC, and glucose have a significant association with outcome in stroke patients.[8] However, most studies were small and did not adjust the blood markers for age and stroke severity in multiple logistic-regression models.[8] None of these markers increased the predictive power of the NIHSS score nor accuracy for long-term outcome.[8]

This study shows that copeptin is a reliable and independent marker to predict long-term outcome in patients with ischemic stroke. Importantly, copeptin improved the prognostic value of the NIHSS score for long-term mortality and functional outcome at 1 year and was a better prognostic marker compared with other markers. Copeptin remained a significant predictor even after adjusting for age and stroke severity and added information for risk stratification beyond the NIHSS score.

Copeptin, though not a specific biomarker, is an attractive tool for routine clinical use because it is easy and quick to measure. In contrast to other brain markers, it directly mirrors intracerebral processes and is released into the systemic circulation, thus bypassing the blood-brain barrier. The strengths of this study are the prospective, consecutive inclusion of well-characterized stoke patients, blinded outcome assessment, and thorough follow-up interviews. As a limitation, our results need validation in an independent cohort of patients.

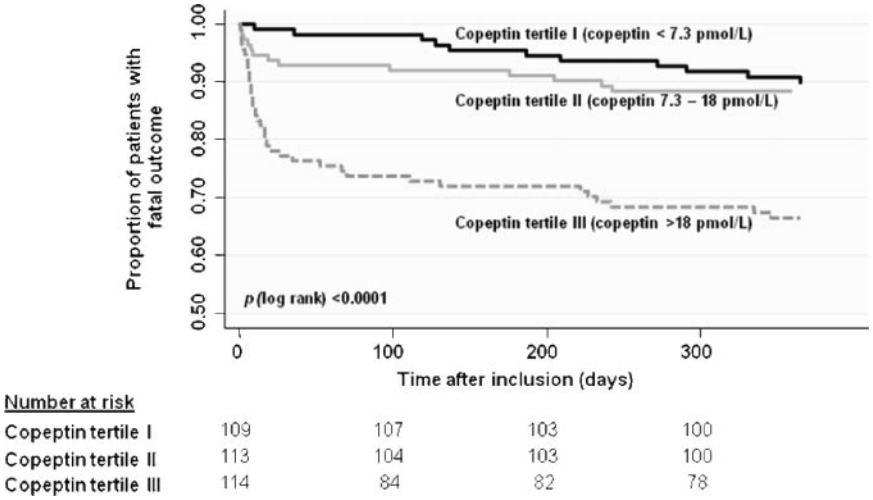

Figure 2. Kaplan–Meier survival curves for copeptin.

In conclusion, we believe that copeptin levels may reliably predict long-term stroke prognosis at its onset. This will allow identification of high-risk patients for whom secondary prevention and intensive rehabilitation can be directed to improve their outcome.

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Disclosures

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Original Article

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Anterior pituitary axis hormones and outcome in acute ischaemic stroke

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Abstract. Neidert S, Katan M, Schuetz P, Fluri F, Ernst A, Bingisser R, Kappos L, Engelter ST, Steck A, Müller B, Christ-Crain M (University Hospital Basel, Basel, Switzerland; SphingoTec GmbH, Borgsorf, Germany; Kantonsspital Aarau, Aarau, Switzerland) Anterior pituitary axis hormones and outcome in acute ischaemic stroke. *J Intern Med* 2011; **269**: 420–432.

Background. Early and accurate prediction of outcome in acute stroke is important and influences risk-optimized therapeutic strategies. Endocrine alterations of the hypothalamic–pituitary axis are amongst the first measurable alterations after cerebral ischaemia. We therefore evaluated the prognostic value of cortisol, triiodothyronine (T3), free thyroxine (fT4), thyroid-stimulating hormone (TSH) and growth hormone (GH) in patients with an acute ischaemic stroke.

Methods. In an observational study including 281 patients with ischaemic stroke, anterior pituitary axis hormones (i.e. cortisol, T3, fT4, TSH and GH) were simultaneously assessed to determine their value to predict functional outcome and mortality within 90 days and 1 year.

Results. In receiver operating characteristic curve analysis, the prognostic accuracy of cortisol was higher compared to all measured hormones and was in the range of the National Institutes of Health Stroke Scale (NIHSS). Cortisol was an independent prognostic marker of functional outcome and death [odds ratio (OR) 1.0 (1.0–1.01) and 1.62 (1.37–1.92), respectively, $P < 0.0002$ for both, adjusted for age and the NIHSS] in patients with ischaemic stroke, but added no significant additional predictive value to the clinical NIHSS score.

Conclusion. Cortisol is an independent prognostic marker for death and functional outcome within 90 days and 1 year in patients with ischaemic stroke. By contrast, other anterior pituitary axis hormones such as peripheral thyroid hormones and GH are only of minor value to predict outcome in stroke.

Keywords: cortisol, hypothalamic–pituitary axis, stroke.

Introduction

Stroke is the third commonest cause of mortality worldwide and a major cause of long-term disability [1]. Stroke strongly influences an individual's emotional and socio-economic quality of life [1]. It is estimated that 795 000 people suffered an acute stroke in the United States of America in 2009, of whom 15–30% remain permanently disabled [2]. An early risk assessment with an estimate of the severity of disease and prognosis is pivotal for optimized care and allocation of healthcare resources. The National Institutes of Health Stroke Scale (NIHSS) is a standardized and widely used assessment measure to predict 3-month outcome in acute cerebrovascular events but its use implies special training and there is inter-observer variability [3]. In this context, rapidly measurable markers to predict illness development, outcome and mortality might improve the prognostic accuracy of clinical scores and traditional risk factors.

The classical 'stress response' of the body occurs after stimulation of the hypothalamic–pituitary axis (HPA axis) by a stressor. It is characterized by an increase in cortisol levels, depression of thyroid function and a functional deficit of anabolic hormones such as growth hormone (GH) and insulin [4]. Anabolic resistance might contribute to the prolonged whole-body protein breakdown with increased susceptibility for infections and delayed recovery. In cerebral ischaemia, endocrine changes of the HPA axis are one of the first measurable alterations [5, 6]. In addition,

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low triiodothyronine (T3) levels, as in the 'low T3 syndrome', have been described as a prognostic risk factor for death and functional outcome in stroke patients [7]. We therefore evaluated the prognostic value of cortisol, T3, free thyroxine (fT4), thyroid-stimulating hormone (TSH) and human GH on hospital admission in a well-described cohort of 281 patients with ischaemic stroke.

Material and methods

Study design and setting

The design of this prospective cohort study at the University Basel, Switzerland (Clinical Trials.gov number, NCT00390962), has been described in detail elsewhere [8, 9]. Briefly, from November 2006 until November 2007, consecutive patients presenting with acute ischaemic stroke were included. Informed consent was obtained from the patient if possible, otherwise from a relative or from the patient's physician in the absence of a relative. This study adheres to the consolidated standards for the reporting of observational trials [10] and was approved by the Ethics Committee of Basel, Switzerland.

Patients

Patients were eligible for the study if they were admitted with acute ischaemic stroke according to the World Health Organization criteria [11], with symptom onset within 72 h.

Of a total of 362 eligible patients, blood was collected on day 1 after admission for standardized measurement of cortisol, thyroid hormones and GH in 281 patients; these measurements could not be performed in 81 patients (two patients died, 68 were discharged to another institution before the standardized blood sampling on the day after admission could be performed and blood sampling was omitted in 11 patients by mistake). However, these 281 patients were similar in terms of baseline characteristics [age ($P = 0.50$), gender ($P = 0.82$), NIHSS ($P = 0.65$) and weight ($P = 0.89$)] compared to the overall cohort. Of the original 281 stroke patients, 268 completed the 1-year follow-up and were available for long-term analysis.

Clinical variables

Within the first 24 h after admission, the following data were recorded: vital signs; relevant co-morbidities assessed by the Charlson comorbidity index (CCI) adjusted for stroke (the CCI is a comorbidity scoring system that includes weighting factors on the basis of disease severity according to the ICD-9-CM system) [12]; medication; traditional risk factors (i.e. age, gender, smoking habits, hypercholesterolaemia, history of hypertension, diabetes mellitus or transient ischaemic attack (TIA)/ischaemic stroke, or positive family history of myocardial infarction, stroke or TIA); and severity of stroke as assessed by the NIHSS [13] by a neurologist certified in the use of this scale. The clinical stroke syndrome was determined by applying the criteria of the Oxfordshire Community Stroke Project: total anterior circulation syndrome (TACS); partial anterior circulation syndrome (PACS); lacunar syndrome (LACS); and posterior circulation syndrome (POCS) [14]. Patients underwent routine laboratory testing and standardized diagnostic work-up to evaluate stroke aetiology. Stroke aetiology was determined according to the criteria of the TOAST classification [15], which distinguishes between large-artery arteriosclerosis, cardioembolism, small-artery occlusion and other or undetermined aetiologies.

Neuroimaging

To exclude intracranial haemorrhage, cranial computed tomography was performed in all patients on admission. Thereafter magnetic resonance imaging (MRI) was performed on a clinical 1.5 T MR Avanto system (SIEMENS, Erlangen, Germany) using a stroke protocol, including T1-, T2- and diffusion-weighted imaging (DWI) sequences, and magnetic resonance angiography. MRI with DWI data was available for 169 patients (60.1%). In these patients, DWI lesion volumes were determined by consensus of two experienced neuroradiologists unaware of the clinical and laboratory results. The lesion size was calculated by the commonly used semi-quantitative method [16]. Lesions were classified into three sizes to represent typical stroke patterns: (i) small lesions with a volume of <10 mL; (ii) medium lesions of 10–100 mL; and (iii) large lesions with a volume of more than 100 mL [17].

Assays

Blood samples were obtained from an indwelling venous catheter the first morning after admission. Routine blood count and C-reactive protein (CRP) levels were measured in all patients. Plasma was collected at the time of blood sampling in plastic tubes containing ethylenediaminetetraacetic acid. The tubes were placed on ice and centrifuged at 3000 ***g***, and plasma was stored at −70 °C until required for assay.

Cortisol was measured with a competitive chemiluminescence immunoassay (IMMULITE 2000; Siemens Medical Solution Diagnostics, Los Angeles, CA, USA) with a calibration range from 28 to 1380 nmol L^{-1}. T3 (nmol L^{-1}), fT4 (pmol L^{-1}) and TSH (mIU L^{-1}) were measured by an electrochemical luminescence immunoassay (Roche Diagnostics, Mannheim, Germany). GH was measured using a high-sensitivity chemiluminescence immunoassay with a functional assay sensitivity of 0.027 ng mL^{-1} as described recently [18]. For all measurements, levels that were not detectable were considered to have a value equal to the lower limit of detection of the assay.

Outcomes

The primary end-point was functional outcome on day 90. It was assessed by two trained medical students, blinded to hormone levels, with a structured follow-up telephone interview with the patient or, if not possible, with the closest relative or family doctor if no close relatives were available. Functional outcome was assessed by the modified Rankin Scale (mRS) [19]. A favourable functional outcome was defined as an mRS of 0–2 points, whereas an unfavourable outcome was defined as an mRS of >2 points. Secondary end-points were all-cause mortality within 90 days, as well as long-term functional outcome and mortality after 1 year.

Statistical analysis

First, to assess the association between hormones and stroke severity, hormone levels were correlated with NIHSS and with lesion size using Spearman's rank correlation. Hormone levels were further assessed for different clinical stroke syndromes, and results are presented as median ± interquartile range (IQR). In addition, hormone levels were compared with regard to primary and secondary end-points. Two-group comparisons were performed with the Mann–Whitney *U*-test and multigroup comparisons with Kruskal–Wallis one-way analysis of variance.

Second, we investigated the association between different hormone levels and both outcomes in univariate logistic regression models, and results are reported as OR. We then adjusted all hormones with significant univariate associations for NIHSS and age, the main outcome predictors within this cohort, as described previously [8]. Further, we performed receiver operating characteristics (ROC) curve analysis to assess discrimination and results are reported as area under the curve (AUC). To study the ability of cortisol to predict mortality, we calculated Kaplan–Meier survival curves and stratified patients by cortisol quartiles. Finally, we calculated reclassification tables [20, 21], and results are reported as net reclassification improvement for outcome and mortality risk categories, as proposed previously [8, 22]. For net reclassification improvement, only those changes in estimated prediction probabilities that imply a change from one risk category to another are considered.

All statistical tests were two-tailed, and $P < 0.05$ was considered to indicate statistical significance. All statistical analysis was performed with medcalc for windows (version 7.2.1.0.; MedCalc, Mariakerke, Belgium), graph pad prism (version 4; GraphPAd, La Jolla, CA, USA) or stata 9.2 (Stata Corp, College Station, TX, USA).

Results

Baseline characteristics of the study population

A total of 281 patients were included in this analysis; 113 (40%) were male and median age was 68 years (IQR 63–82). Median systolic blood pressure was 160 mmHg (IQR 141–180), median diastolic blood pressure was 90 mmHg (IQR 80–100) and median heart rate was 78 beats min^{-1} (IQR 68–88). A total of 58 (21%) patients were diagnosed with atrial fibrillation, 74 (26%) patients had hypercholesterolaemia, 84 (30%) had a positive family history of cardiovascular events and 98 (35%) were smokers. Median glucose levels were 6.1 mmol L^{-1} (IQR 5.4–7.4). The main baseline characteristics are summarized in Table 1.

Pituitary axis hormones and stroke characteristics

NIHSS. There was a positive correlation between the NIHSS and levels of cortisol ($r = 0.32$, $P < 0.0001$) and GH ($r = 0.15$, $P = 0.004$), and a negative correlation with levels of T3 ($r = -0.27$, $P < 0.0001$) and TSH ($r = -0.17$, $P = 0.006$). fT4 levels were not correlated with the NIHSS ($r = -0.0008$, $P = 0.78$) (Fig. 1).

Lesion size. In patients for whom MRI data were available ($n = 169$), cortisol levels increased with lesion size. Median cortisol levels in patients with small, medium and large lesions were 461 nmol L^{-1} (IQR 345–585), 490 nmol L^{-1} (IQR 367–631) and 749 nmol L^{-1} (IQR 648–631), respectively ($P < 0.0001$). There was no difference in T3 or fT4 levels with lesion size. However, TSH levels were lower in

Table 1 *Baseline characteristics of stroke patients*

Demographic characteristics	
Age (years) median (IQR)	68 (63–82)
Male sex (%)	59
Clinical findings median (IQR)	
Heart rate (beats min^{-1})	78 (68–88)
Systolic blood pressure (mmHg)	160 (141–180)
Diastolic blood pressure (mmHg)	90 (80–100)
Temperature (°C)	37.0 (36.5–37.5)
Weight (kg)	72 (64–82)
Height (cm)	169 (162–175)
Body mass index (BMI) (kg m^{-2})	25.2 (24–27.2)
Laboratory findings (median–IQR)	
Cortisol (nmol L^{-1}) (n = 281)	480 (344.5–629.8)
T3 (nmol L^{-1}) (n = 269)	1.4 (1.2–1.6)
fT4 (pmol L^{-1}) (n = 274)	15.4 (13.8–17.2)
TSH (mIU L^{-1}) (n = 275)	1.4 (0.9–2.2)
Growth hormone (ng mL^{-1}) (n = 276)	0.4 (0.2–1.1)
Total cholesterol (mmol L^{-1}) (n = 268)	4.4 (3.8–5.1)
High-density lipoproteins (HDL) (mmol L^{-1}) (n = 281)	1.3 (1.1–1.6)
Low-density lipoproteins (LDL) (mmol L^{-1}) (n = 281)	2.4 (1.8–3.0)
Triglycerides (mmol L^{-1}) (n = 281)	1.1 (0.9–1.6)
C-reactive protein (mg L^{-1}) (n = 281)	3.4 (3.0–9.4)
Glucose (mmol L^{-1}) (n = 281)	6.1 (5.4–7.4)
Prognostic scores (median–IQR)	
Modified ranking scale (points)	2 (1–4)
NIHSS (points)	5 (2–10)
Stroke syndrome no. (%)	
TACS	27 (9.4)
PACS	126 (43.8)
LACS	58 (20.1)
POCS	71 (24.7)
Stroke aetiology no. (%)	
Small-vessel occlusive	48 (16.7)
Large-vessel occlusive	54 (18.8)
Cardioembolic	105 (36.5)
Other	13 (4.5)
Unknown	61 (21.2)
Vascular risk factors no. (%)	
Hypertension	214 (74)
Atrial fibrillation	58 (20)
Smoking history	98 (34)
Hypercholesterolaemia	74 (26)
Diabetes mellitus	56 (19)
Coronary heart disease	67 (23)
Prior stroke	66 (23)
Family history of cardiovascular event	84 (29)

fT4, free thyroxine; IQR, interquartile range; LACS, lacunar syndrome; NIHSS, National Institutes of Health Stroke Scale; PACS, partial anterior circulation syndrome; POCS, posterior circulation syndrome; T3, triiodothyronine; TACS, total anterior circulation syndrome; TSH, thyroid-stimulating hormone.

patients with large lesions compared to those with small lesion [0.62 mIU L^{-1} (IQR 0.3–1.1) vs. 1.33 mIU L^{-1} (IQR 1.1–1.6), $P < 0.01$], and GH levels increased with lesion size [0.21 ng mL^{-1} (IQR 0.10–0.94) vs. 0.57 ng mL^{-1} (IQR 0.16–1.21) vs. 1.65 ng mL^{-1} (IQR 0.84–2.40), overall $P = 0.003$].

Clinical stroke syndrome. Cortisol values were significantly higher in patients with TACS 654 nmol L^{-1} (IQR 495–839) compared with patients with PACS 472 nmol L^{-1} (IQR 328–624, $P < 0.001$), LACS 450 nmol L^{-1} (IQR 334–587, $P < 0.001$) or POCS 469 nmol L^{-1} (IQR 351–613, $P < 0.01$). Thyroid hormone levels did not vary in patients with different clinical stroke syndromes. GH levels were significantly higher in patients with TACS [0.59 ng mL^{-1} (IQR 0.36–1.06)] compared to those with LACS [0.31 ng mL^{-1} (IQR 0.11–0.84), $P < 0.05$] and tended to be higher compared to those with PACS or POCS [0.35 ng mL^{-1} (IQR 0.15–1.03) and 0.43 ng mL^{-1} (IQR 0.14–1.22), respectively, $P = 0.05$].

Pituitary axis hormones and outcome

Pituitary axis hormones and 90-day outcome. A total of 172 (61%) patients had a good functional outcome defined as an mRS ≤ 2, whereas 109 patients (39%) had a bad functional outcome defined by an mRS ≥ 3.Thirty patients died within 90 days, and thus the mortality rate was 10.7%. The comparisons of median hormone values in patients with a good/-bad functional outcome and in survivors/nonsurvivors are summarized in Table 2.

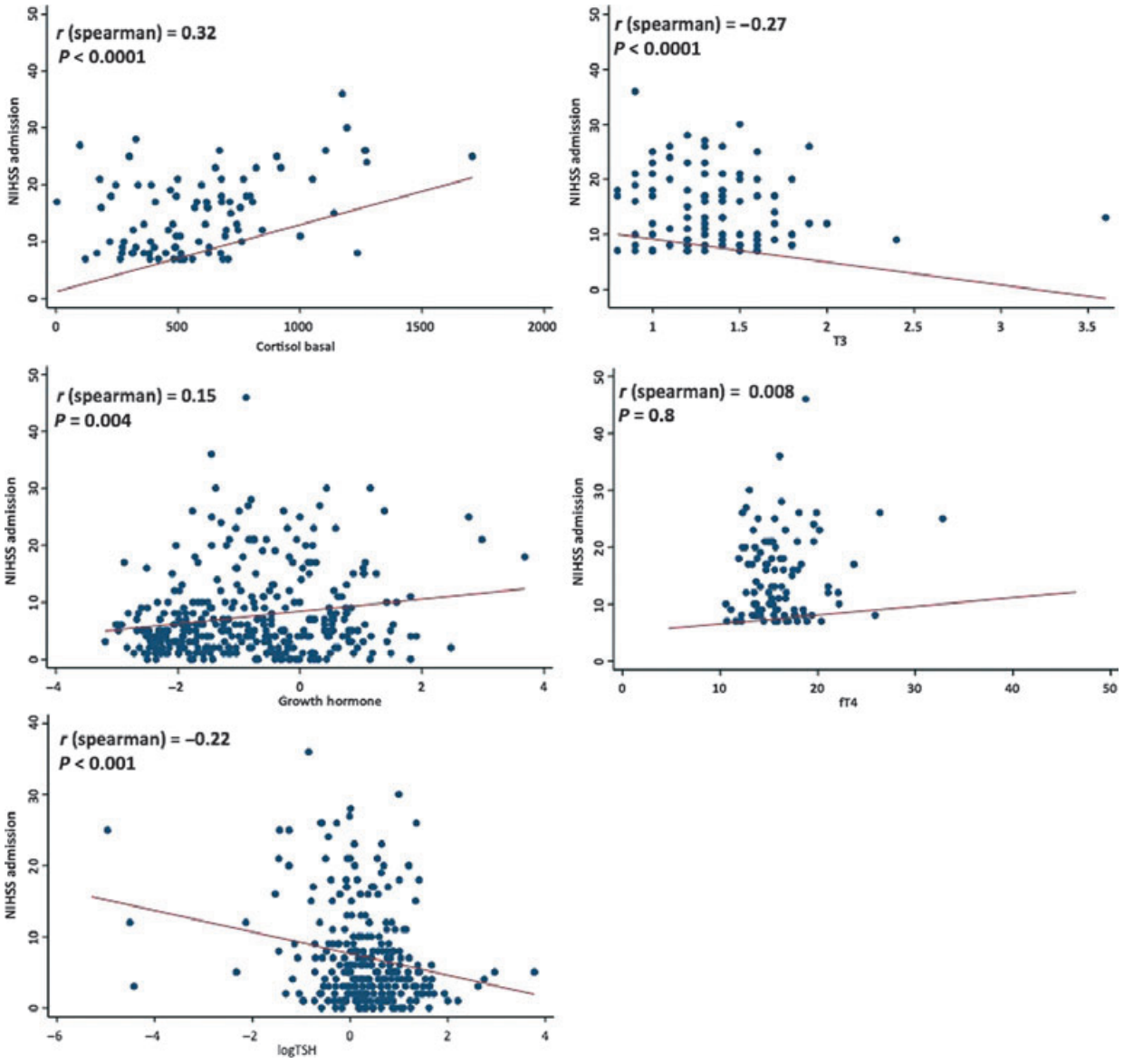

Fig. 1 *Correlation between the National Institutes of Health Stroke Scale (NIHSS) and pituitary axis hormone levels. Rank correlation between the NIHSS and levels of cortisol, growth hormone, triiodothyronine, free thyroxine and thyroid-stimulating hormone.*

Cortisol. Median cortisol levels in patients with a favourable outcome and in survivors were lower compared to levels in patients with an unfavourable outcome and nonsurvivors, respectively.

Time to death was analysed by Kaplan–Meier survival curves based on cortisol quartiles. Patients in the highest two quartiles (cortisol ≥ 633 nmol L^{-1}) had an increased risk of death compared with patients in the lowest two quartiles ($P < 0.001$) (Fig. 2).

T3, fT4 and TSH. Triiodothyronine levels in stroke patients with a favourable outcome were higher compared to levels in patients with an unfavourable outcome; the reverse was found for fT4 levels. T3/fT4 ratios were significantly higher in patients with a

Table 2 *Pituitary axis hormone levels and outcome after 90 days and 1 year*

	Functional outcome			Mortality		
	mRS ≤ 2	mRS > 2	*P*	Survivors	Nonsurvivors	*P*
Pituitary axis hormones and 90-day outcome						
Cortisol ($mmol\ L^{-1}$) median (IQR)	444 (318.5–558.5)	582 (439.5–727)	<0.0001	466 (337–598)	712 (577–1106)	<0.0001
T3 ($nmol\ L^{-1}$) median (IQR)	1.5 (1.3–1.6)	1.3 (1.1–1.6)	0.005	1.5 (1.3–1.6)	1.2 (1.0–1.4)	0.002
fT4 ($pmol\ L^{-1}$) median (IQR)	15.6 (14.2–17.8)	15.3 (13.7–16.6)	0.03	15.4 (13.8–16.9)	16.3 (14.3–19.6)	0.03
T3/fT4 ratio	0.1 (0.08–0.1)	0.08 (0.07–0.1)	0.0001	0.09 (0.08–0.11)	0.07 (0.06–0.08)	0.0001
TSH ($mIU\ L^{-1}$) median (IQR)	1.6 (1.1–2.4)	1.1 (0.7–2.0)	0.0003	1.4 (0.9–2.3)	1.0 (0.6–2.2)	0.02
GH ($ng\ mL^{-1}$) median (IQR)	0.3 (0.1–1)	0.5 (0.2–1.2)	0.01	0.3 (0.2–1.0)	0.6 (0.4–1.4)	0.02
Pituitary axis hormones and 1-year outcome						
Cortisol ($mmol\ L^{-1}$) median (IQR)	466 (333–585)	512 (377–692)	0.004	466 (337–590)	633 (455–853)	<0.0001
T3 ($nmol\ L^{-1}$) median (IQR)	1.5 (1.3–1.7)	1.3 (1.2–1.7)	0.0008	1.5 (1.3–1.6)	1.3 (1.1–1.6)	0.06
fT4 ($pmol\ L^{-1}$) median (IQR)	15.4 (13.8–16.8)	15.6 (14.1–17.8)	0.11	15.4 (13.8–16.7)	17.1(14.3–19.1)	0.004
T3/fT4 ratio	0.1 (0.08–0.11)	0.08 (0.07–0.1)	0.0002	0.09 (0.08–0.11)	0.08 (0.06–0.09)	0.001
TSH ($mIU\ L^{-1}$) median (IQR)	1.4 (1.0–2.2)	1.2 (0.7–2.1)	0.04	1.4 (0.9–2.2)	1.1 (0.6–2.2)	0.1
GH ($ng\ mL^{-1}$) median (IQR)	0.3 (0.1–1.1)	0.5 (0.2–1.1)	0.06	0.3 (0.1–1.0)	0.6 (0.4–1.5)	0.002

fT4, free thyroxine; GH, growth hormone; IQR, interquartile range; mRS, modified Rankin Scale; T3, triiodothyronine; TSH, thyroid-stimulating hormone.

favourable outcome compared to ratios in patients with an unfavourable outcome. TSH levels were higher in patients with a favourable outcome compared to levels in patients with an unfavourable outcome.

Patients who died had lower T3/TSH levels and higher fT4 levels compared to levels in patients who survived. T3/fT4 ratios were significantly higher in patients who survived compared to ratios in those who died.

Time to death was analysed by Kaplan–Meier survival curves based on fT4, T3 and TSH quartiles. Patients in the highest quartile of fT4 ($P = 0.02$) and the lowest

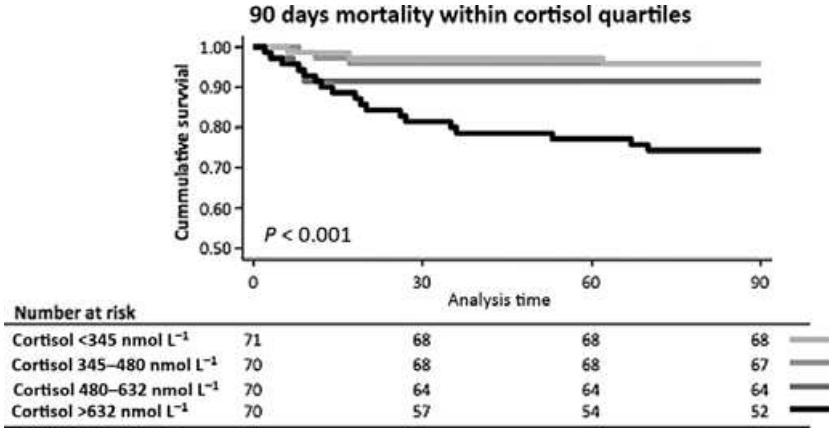

Fig. 2 *Kaplan–Meier survival based on cortisol quartiles. Time to death was analysed by Kaplan–Meier curves based on cortisol quartiles. Patients in the lower two quartiles (cortisol <345 nmol L^{-1} and cortisol between 345 and 480 nmol L^{-1}) had a minor risk of death compared to patients with cortisol levels in the upper two quartile (cortisol between 481 and 632 and ≥633 nmol L^{-1}, $P < 0.001$).*

quartile of T3 ($P = 0.002$) and TSH ($P = 0.01$) had a significantly higher risk of death compared to patients in the other three quartiles.

GH. Growth hormone levels in patients with a favourable outcome and survivors were lower compared to levels in patients with an unfavourable outcome and nonsurvivors, respectively.

Time to death was analysed by Kaplan–Meier survival curves based on GH quartiles. Patients in the highest two quartiles had a significantly increased risk of death compared to patients in the lower two quartiles ($P = 0.001$).

Pituitary axis hormones and outcome at 1 year. Of the original 281 stroke patients, 268 completed the 1-year follow-up and were available for long-term analysis. The comparisons of median hormone values in patients with a good/bad functional outcome and in survivors/nonsurvivors are summarized in Table 2.

Comparison of the pituitary axis hormones with other markers and clinical scores. We performed ROC curve analysis to compare the overall prognostic accuracy of the NIHSS, cortisol, T3, fT4, TSH and GH. The prognostic accuracy of cortisol was high compared to that of glucose and white blood cell count, but was similar to that of CRP and the CCI. The NIHSS was the most accurate predictor of 1-year outcome (Table 3).

The AUC to predict mortality was highest for cortisol [0.81 (IQR 0.76–0.86)] and was similar to that of the NIHSS [0.85 (IQR 0.8–0.89), $P = 0.44$]. Cortisol had a higher prognostic accuracy than the other hormones and laboratory and clinical parameters and tended to have a higher AUC value compared to CRP (Table 3). Again, the NIHSS was the most accurate predictor of mortality at 1 year (Table 3).

When combining the NIHSS with initial cortisol in a logistic regression model for 90-day outcome, we found only a small increase in AUC from 0.75 to 0.77 for functional outcome prediction, which did not reach statistical significance ($P = 0.52$). Similarly, for mortality prediction, the AUC increased from 0.85 to 0.87 ($P = 0.30$).

We further calculated in-sample reclassification tables (Appendix 1 and 2). In patients with poor outcome, 14 were classified in higher-risk categories and 10 in lower categories when using the model with the NIHSS and cortisol. Similarly, in patients with good outcome, only 14 were classified in higher-risk categories and 32 in lower categories. Thus, the estimated net reclassification improvement for functional outcome was 0.14 ($P < 0.01$). Amongst nonsurvivors, seven patients were classified in higher-risk categories and three in lower categories; amongst survivors, 99 patients were classified in lower-risk categories and 50 in higher categories when using the model with the NIHSS score and cortisol (net reclassification improvement 0.33, $P < 0.001$).

Table 3 *Receiver operating characteristics curve analysis*

	Functional outcome at 90 days			Functional outcome at 1 year		
Parameter	AUC	95% confidence interval	*P*	AUC	95% confidence interval	*P*
Cortisol	0.68	(0.63–0.74)		0.60	(0.53–0.66)	
NIHSS	0.75	(0.69–0.8)	0.08	0.72	(0.66–0.78)	0.004
T3	0.56	(0.50–0.63)	0.06	0.61	(0.55–0.67)	0.71
fT4	0.58	(0.52–0.64)	0.03	0.55	(0.49–0.62)	0.38
TSH	0.60	(0.55–0.67)	0.29	0.55	(0.49–0.61)	0.35
GH	0.61	(0.55–0.67)	0.12	0.57	(0.50–0.63)	0.56
Glucose	0.55	(0.49–0.61)	0.01	0.50	(0.44–0.58)	0.05
WBC	0.56	(0.50–0.62)	0.02	0.53	(0.49–0.60)	0.18
CCI	0.62	(0.56–0.68)	0.26	0.64	(0.57–0.71)	0.45
CRP	0.60	(0.54–0.67)	0.16	0.59	(0.52–0.66)	0.84
	Mortality at 90 days			Mortality at 1 year		
Cortisol	0.81	(0.76–0.86)		0.69	(0.63–0.75)	
NIHSS	0.85	(0.80–0.89)	0.44	0.78	(0.72–0.83)	0.11
T3	0.66	(0.60–0.70)	0.02	0.59	(0.52–0.65)	0.10
fT4	0.60	(0.54–0.76)	0.003	0.63	(0.57–0.68)	0.29
TSH	0.60	(0.54–0.67)	0.001	0.56	(0.50–0.62)	0.03
GH	0.64	(0.58–0.70)	0.04	0.65	(0.58–0.71)	0.50
Glucose	0.59	(0.53–0.66)	0.002	0.55	(0.48–0.62)	0.0.2
WBC	0.66	(0.53–0.67)	0.004	0.53	(0.47–0.61)	0.01
CCI	0.59	(0.53–0.65)	0.007	0.59	(0.52–0.66)	0.08
CRP	0.69	(0.63–0.75)	0.13	0.64	(0.57–0.71)	0.27

AUC, area under the curve; CCI, Charlson comorbidity index; CRP, C-reactive protein; fT4, free thyroxine; GH, growth hormone; NIHSS, National Institutes of Health Stroke Scale; T3, triiodothyronine; TSH, thyroid-stimulating hormone; WBC, white blood count.

Association between hormones and both functional outcome and mortality in logistic regression analysis

Univariate logistic regression models showed that cortisol, T3 and TSH were associated with functional outcome. Cortisol, fT4 and T3 were also significantly associated with death (Table 2). In a logistic model adjusted for the NIHSS and age, cortisol, but not the other pituitary axis hormones, was independently associated with both functional outcome [OR 1.0 (1.00–1.01), $P < 0.0002$] and death [OR 1.62 (1.37–1.92), $P < 0.0002$] (Table 4).

Discussion

In this study, we simultaneously assessed anterior pituitary axis hormones with regard to their accuracy to predict functional outcome and mortality in patients with acute ischaemic stroke within 90 days and 1 year. Our main finding is that cortisol is an independent prognostic marker of functional outcome and death in patients with ischaemic stroke, but adds no significant additional predictive information to the clinical score of the NIHSS. We demonstrated that cortisol levels increased with lesion size, neurological deficit (assessed by the NIHSS) and the clinical stroke syndrome (i.e. TACS versus PACS, LACS and POCS), reflecting the severity of the stroke. Conversely, T3, fT4, TSH and GH add only limited or no prognostic information to currently used measures and scores.

In previous studies, we found that copeptin is a significant predictor of short- and long-term outcome and mortality [8, 9]. The prognostic performance of cortisol within the same population was similar to that of copeptin, but showed no significant additional predictive value to the NIHSS in contrast to copeptin [8].

Table 4 *Univariate and multivariate association between hormone levels and outcome*

	Univariate analysis			Multivariate analysis		
Parameter	Odds ratio	$P > z$	95% confidence interval	Odds ratio	$P > z$	95% confidence interval
Predictor: functional outcome						
Cortisol (per 100 nmol increase)	1.00	<0.0002	(1.00–1.00)	1.23	<0.01	(1.07–1.43)
fT4	1.07	0.08	(0.99–1.14)			
T3	0.32	0.01	(0.15–0.72)	0.84	0.09	(0.68–1.02)
TSH	0.78	0.01	(0.63–0.95)	0.79	0.60	(0.33–1.90)
GH	0.99	0.81	(0.90–1.08)			
Predictor: death						
Cortisol (per 100 nmol increase)	1.62	<0.0002	(1.37–1.92)	1.43	<0.001	(1.17–1.75)
fT4	1.10	0.02	(1.01–1.20)	1.08	0.13	(0.98–1.19)
T3	0.19	0.01	(0.05–0.71)	0.69	0.66	(0.13–3.60)
TSH	0.92	0.50	(0.72–1.17)			
GH	1.04	0.41	(0.95–1.14)			

Multivariate analysis was calculated for all significant predictors in univariate analysis, adjusted for age and the National Institutes of Health Stroke Scale.
fT4, free thyroxine; GH, growth hormone; T3, triiodothyronine; TSH, thyroid-stimulating hormone.

Acute ischaemic stroke acts as a stressor and thus stimulates the HPA axis resulting in increased glucocorticoid levels [5, 6, 23]. The higher cortisol levels observed in patients with worse functional outcome or subsequent death reflect a higher degree of stress. These results are in accordance with the results from other studies showing that serum cortisol levels rise proportionally with the degree of stress and correlate with stroke severity [5, 24–26]. A severe stroke per se implies a poor outcome. However, there are several other mechanisms that might explain the unfavourable outcome in patients with higher cortisol levels. Hypercortisolism has been suggested to potentiate ischaemic neuronal injury, especially in hippocampal neurons [27], and the corticosterone synthesis inhibitor metyrapone was able to prevent ischaemia-induced loss of synaptic function in the hippocampus of rats [28]. The hippocampus has an important role in the feedback regulation of the HPA axis. A disturbed hippocampus function might result in a false HPA axis feedback that potentiates hypercortisolism and causes a vicious circle, explaining the worse prognosis in stroke patients with high cortisol levels [29]. In addition, patients with stroke and high cortisol levels have been shown to be more prone to adverse cardiac events (e.g. arrhythmias or myofibrillar degeneration), which might lead to higher mortality rates [25, 30]. Another major cause of a bad prognosis after stroke is the development of infectious disease which is related to an immune dysregulation resulting from neuroendocrine disturbance after stroke.

In our study, T3 levels were lower in patients with a poorer prognosis concerning functional outcome and death. This is in line with a previously published study which showed that the low T3 syndrome was an independent predictor of survival in patients with acute stroke, and predicted disability at 1 year [7]. It should be emphasized that low T3 levels even within the normal range are already associated with poorer prognosis in acute stroke patients [7]; the greater the severity of disease, the lower the serum T3 levels. A decrease in the peripheral production of T3 because of decreased extra-thyroidal conversion of T4 into T3 by the enzyme type I iodothyronine-5′-deiodinase, as in the low T3 syndrome, is a major contributing factor [31]. This is reflected by the lower T3/fT4 ratios in patients with an unfavourable outcome in our cohort. In addition, it has been shown that high levels of corticosteroids suppress TSH secretion and the pituitary response to thyrotropin-releasing hormone in man [32], leading, for example, to low T3 levels. Furthermore, stress-induced elevation of glucocorticoids in rats causes suppression of TSH and T3 levels [33]. Thus, the higher glucocorticoid levels in patients with worse outcome measured in our cohort might result in a higher decrease in T3 levels.

Growth hormone levels in our study were higher in patients who died than in those who survived. Several mechanisms might explain the poorer prognosis of patients with higher GH levels. First, GH levels increase during stress and thus mirror the stress

associated with the severity and extent of illness [4]. Second, the higher levels of GH in nonsurvivors might be an attempt by the body to provide energy and postpone anabolism. Third, the higher GH levels in our study observed in nonsurvivors might be related to lower insulin-like growth factor (IGF) levels and reflect hepatic GH resistance. In this context, it has been shown that patients with lower IGF levels suffering from an ischaemic or haemorrhagic stroke are at higher risk of death compared to patients with higher IGF levels [34, 35]. IGF is a potent neurotrophic factor. Its expression is induced in injured brain regions, and its administration reduces the extent of cortical infarction and neuronal death from ischaemic injury in animal models [36].

Limitations

Some limitations of this observational study merit consideration. First, cortisol improved the classification of patients for functional outcome and for death in net reclassification statistics as evidenced by significant net reclassification improvements [21]. However, the lack of significance in the combined ROC curve analysis suggests that these findings need to be interpreted with caution. For this reason, we did not further determine cut-off values for cortisol for use in clinical practice.

Second, we only performed single measurements of hormone levels in the morning after admission. GH and cortisol levels show a large variation during the day because in terms of GH, secretion is pulsatile [37] with almost undetectable serum GH concentrations between the pulses, and in terms of cortisol, it follows a circadian rhythm [38]. A standardized measurement of GH with a functional test in combination with IGF measurement in all patients might have provided a higher accuracy to predict outcome. For cortisol, however, the confounding factors are minimized as the acute illness abolishes its diurnal variation and as we similarly measured the level in the morning after admission in all patients [39]. Also, standardized measurements do not represent clinical routine in the acute emergency room setting.

Third, we analysed patients within 72 h of symptom onset. When comparing initial results with results from hormone levels of patients the next morning after admission and within either 12–24 or 24–72 h after symptom onset, they did not show significant differences. We also tested for effect modification by delay to blood sampling for cortisol measurement and did not find evidence for interaction (between cortisol levels and time delay) with regard to prediction of death and functional outcome (data not shown).

Conclusion

In conclusion, in this study, we simultaneously assessed the prognostic accuracy of pituitary axis hormones (i.e. cortisol, T3, fT4 and GH) in a large cohort of patients with acute ischaemic stroke. We found that cortisol levels and, to a lesser extent, T3, fT4 and GH levels mirror stroke severity. Cortisol levels are independently associated with an unfavourable outcome after acute ischaemic stroke.

Conflict of interest statement

AE was an employee of SphingoTec GmbH, the developer of the GH assay. No funding was obtained from commercial sources for this study.

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Appendix 1 *Reclassification table for functional outcome*

	Model with NIHSS and cortisol							
Model with NIHSS	<3% risk	3% to <10% risk	10% to <18% risk	18% to <35% risk	35% to <40% risk	40% to 48% risk	>48% risk	Total no.
Patients with good functional outcome								
<3% risk	0	0	0	0	0	0	0	0
3% to <10 % risk	0	0	0	0	0	0	0	0
10% to <18% risk	0	1	13	1	0	0	0	15
18% to <35% risk	0	3	22	84	8	0	0	117
35% to <40% risk	0	0	0	0	6	3	0	9
40% to 48% risk	0	0	0	0	3	5	2	10
>48% risk	0	0	0	0	0	3	17	20
Total no.	0	4	35	85	17	11	19	171
Patients with bad functional outcome								
<3% risk	0	0	0	0	0	0	0	0
3% to <10 % risk	0	0	0	0	0	3	0	0
10% to <18% risk	0	0	3	0	0	0	0	3
18% to <35% risk	0	0	1	29	2	3	0	35
35% to <40% risk	0	0	0	2	1	2	0	5
40% to 48% risk	0	0	0	2	3	5	4	14
>48% risk	0	0	1	0	1	0	50	52
Total no.	0	0	5	33	7	10	54	109

Appendix 2 *Reclassification table for mortality*

	Model with NIHSS and cortisol							
Model with NIHSS	<1% risk	1% to <2% risk	2% to <4% risk	4% to <9% risk	9% to <18% risk	18% to 34% risk	>34% risk	Total no.
Survivors								
<1% risk	0	0	0	0	0	0	0	0
1% to <2% risk	0	0	0	0	0	0	0	0
2% to <4% risk	24	33	44	28	0	1	0	130
4% to <9% risk	2	7	14	31	13	2	0	69
9% to <18% risk	0	1	1	5	10	3	0	20
18% to 34% risk	1	0	1	1	2	11	3	19
>34% risk	0	0	1	2	2	2	5	12
Total no.	27	41	61	67	27	19	8	250
Nonsurvivors								
<1% risk	0	0	0	0	0	0	0	0
1% to <2% risk	0	0	0	0	0	0	0	0
2% to <4% risk	0	0	1	1	0	0	0	2
4% to <9% risk	0	0	2	3	1	1	0	7

Appendix 2 *(Continued)*

	Model with NIHSS and cortisol							
Model with NIHSS	<1% risk	1% to <2% risk	2% to <4% risk	4% to <9% risk	9% to <18% risk	18% to 34% risk	>34% risk	Total no.
9% to <18% risk	0	0	0	0	3	2	0	5
18% to 34% risk	0	0	0	0	0	2	2	4
>34% risk	0	0	0	0	0	1	11	12
Total no.	0	0	3	4	4	6	13	30

Copeptin adds prognostic information after ischemic stroke

Results from the CoRisk study

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ABSTRACT

Objective: To evaluate and validate the incremental value of copeptin in the prediction of outcome and complications as compared with established clinical variables.

Methods: In this prospective, multicenter, cohort study, we measured copeptin in the emergency room within 24 hours from symptom onset in 783 patients with acute ischemic stroke. The 2 primary end points were unfavorable functional outcome (modified Rankin Scale score 3–6) and mortality within 90 days. Secondary end points were any of 5 prespecified complications during hospitalization.

Results: In multivariate analysis, higher copeptin independently predicted unfavorable outcome (adjusted odds ratio 2.17 for any 10-fold copeptin increase [95% confidence interval {CI}, 1.46–3.22], $p < 0.001$), mortality (adjusted hazard ratio 2.40 for any 10-fold copeptin increase [95% CI, 1.60–3.60], $p < 0.001$), and complications (adjusted odds ratio 1.93 for any 10-fold copeptin increase [95% CI, 1.33–2.80], $p = 0.001$). The discriminatory accuracy, calculated with the area under the receiver operating characteristic curve, improved significantly for all end points when adding copeptin to the NIH Stroke Scale score and the multivariate models. Moreover, the combination of copeptin with a validated score encompassing both the NIH Stroke Scale and age led to a net reclassification improvement of 11.8% for functional outcome and of 37.2% for mortality.

Conclusions: In patients with ischemic stroke, copeptin is a validated blood marker that adds predictive information for functional outcome and mortality at 3 months beyond stroke severity and age. Copeptin seems to be a promising new blood marker for prediction of in-hospital complications. *Neurology*® 2013;80:1–9

GLOSSARY

AUC = area under the curve; **AVP** = arginine vasopressin; **CI** = confidence interval; **CRP** = C-reactive protein; **HR** = hazard ratio; **mRS** = modified Rankin Scale; **IQR** = interquartile range; **NIHSS** = NIH Stroke Scale; **NRI** = net reclassification improvement; **OR** = odds ratio; **ROC** = receiver operating characteristic.

Accurate and prompt prediction of functional outcome, mortality, and complications in patients with ischemic stroke is essential for patients, families, and clinicians. In this context, rapidly measurable and reliable blood biomarkers may refine clinical decision-making. Several blood biomarkers have shown the potential to predict outcome after ischemic stroke. However, to be useful in clinical routine, blood biomarkers are expected to improve the prognostic accuracy of established clinical variables such as stroke severity and age.[1] At present, the investigated biomarkers have either failed to further improve prognostication after stroke, or they have not been studied for their additional prognostic value.[1,2] However, a recent single-center study showed that copeptin, a hypothalamic hormone derived from the precursor of vasopressin, predicted outcome and mortality 3 months and 1 year after ischemic stroke, improving the prognostic accuracy of established predictors.[3,4]

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Supplemental data at www.neurology.org

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Copeptin is a reliable prognostic marker not only in stroke patients but also in patients with cardiovascular events.[5,6] However, before implementing copeptin in clinical practice, the prognostic potential of copeptin needs to be validated in a prospective, independent, large, multicenter study. Moreover, it is unclear whether copeptin predicts outcome in patients with ischemic stroke that has been treated differently, i.e., conservatively or with thrombolysis. The CoRisk study aimed to validate in a multicenter, international setting the accuracy of copeptin in predicting functional outcome, mortality, and complications as compared with established clinical variables.

METHODS

Ethics statement. This study (ClinicalTrials.gov: unique identifier NCT00878813, http://www.clinicaltrials.gov/ct2/show/NCT00878813) was conducted according to the principles expressed in the Declaration of Helsinki and it was approved by the Ethics Committees. All patients or their welfare guardians provided written informed consent for the collection of data, blood samples, and subsequent analyses.

Study design and cohort description. The primary design of this multicenter, prospective, cohort study has been described in detail previously.[7] For the analysis of this study, we included 788 patients older than 18 years with an acute ischemic stroke within 24 hours of symptom onset, admitted consecutively to the emergency department of each tertiary care center between March 24, 2009 and April 8, 2011.

We defined acute ischemic stroke according to the World Health Organization criteria as an acute focal neurologic deficit lasting longer than 24 hours[8] with no sign of acute intracranial bleeding on cerebral imaging. Exclusion criteria were missing informed consent or any diagnosis different from ischemic stroke (i.e., stroke mimics). Stroke physicians prospectively recorded the NIH Stroke Scale (NIHSS)[9] score upon admission. The clinical stroke syndrome was assessed according to the Oxfordshire Community Stroke Project classification.[10] CT or MRI was performed upon admission. MRI with diffusion-weighted imaging was performed in 537 stroke patients. Diffusion-weighted imaging lesion volumes were measured by the consensus of 2 experienced raters unaware of the clinical and laboratory findings. The lesion size was calculated by a frequently used semiquantitative method validated for ischemic stroke lesions.[11] Lesions were categorized into 3 size classes to represent typical stroke patterns: 1) small lesion with a volume of <10 mm^3, 2) medium lesion of 10–100 mm^3, and 3) large lesion with a volume of >100 mm^3.[3]

Detailed information such as cardiac and neurovascular ultrasound and 24-hour EKGs were collected to define stroke etiology according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification.[12] Comorbidities were assessed on admission by the modified Charlson Comorbidity Index.[13]

Biomarker measurement. For all patients, blood was drawn in the emergency room and within 24 hours of symptom onset. After centrifugation for 20 minutes at 3,000*g* at room temperature, plasma (from EDTA tube) was aliquoted. Tubes were frozen locally at each center at −70°C. Copeptin levels were assessed in plasma in a blinded batch analysis by a new chemiluminescence sandwich immunoassay. The lower detection limit was 0.4 pmol/L and the functional assay sensitivity was <1 pmol/L ($<20\%$ interassay coefficient of variation, defined as the ratio of the SD to the mean). In 359 healthy individuals, median copeptin levels were reported to be 4.2 pmol/L with a 99th percentile of 13.5 pmol/L.[14]

Ascertainment of outcomes. Trained stroke physicians and study nurses assessed outcome 3 months after the acute stroke, either during an outpatient visit (patients who underwent thrombolysis) or with a structured follow-up telephone interview. They were blinded to copeptin levels and baseline clinical variables.

Primary end points. The 2 primary end points were 1) unfavorable functional outcome (including mortality) defined as a modified Rankin Scale (mRS) score of 3 to 6, and 2) mortality within 90 days of hospital admission.

Secondary end point. The secondary outcome included any of the following prespecified[7] complications during hospital stay: symptomatic intracerebral hemorrhage according to the Safe Implementation of Thrombolysis in Stroke-Monitoring Study criteria,[15] space-occupying cerebral edema, pneumonia (defined as auscultatory respiratory crackles combined with body temperature $\geq$38°C, purulent sputum, or positive chest radiograph), seizures (clinical diagnosis of focal and/or generalized seizure in a previously nonepileptic patient), or mortality within 10 days from stroke onset.

Statistical analysis. ***Univariate analysis.*** Statistical analysis was performed for the primary and secondary end points separately. Discrete variables were expressed as counts (percentages) and continuous variables as means ± SD or medians (interquartile range [IQR]), depending on their distribution. The distribution of raw biomarker data was skewed. After log transformation with a base of 10, the distribution of the biomarker data approximated a normal distribution. Comparisons for categorical baseline measurements were performed by Fisher exact test and for continuous, not normally distributed baseline data, by the Mann-Whitney *U* test. For survival analysis, we stratified patients according to copeptin tertiles in Kaplan-Meier curves and compared the groups by means of the log-rank test.

Multivariate regression models. To assess the independent association of copeptin with functional outcome and time to fatal outcome within 3 months, we computed a multivariate logistic and Cox regression model, respectively. We prespecified that models were adjusted for NIHSS score, age, lesion size, modified Charlson Index,[13] and total anterior circulation stroke[10] based on the results of the previous derivation study.[3] In addition, the final multivariate models included variables significantly associated with an unfavorable outcome or mortality in the univariate analyses. Because patients with severe stroke tend to present earlier, time from symptom onset to blood collection was included in the final model.[16] We report odds ratios (ORs) and hazard ratios (HRs) along with 95% confidence intervals (CIs) as measure of association and uncertainty, respectively. OR and HR correspond to a 1-unit increase in the explanatory variable and to any 10-fold increase in copeptin, glucose, or C-reactive protein (CRP) levels (log-transformed with a base of 10). Goodness-of-fit of the multivariate logistic and Cox regression models were assessed with the Hosmer-Lemeshow test and Groennesby and Borgan test, respectively.

Interaction analysis. We further included interaction terms to investigate whether the predictive value of copeptin is modified by treatment status (conservative vs thrombolysis), sex, age, stroke severity (NIHSS scores 0–6, 7–15, and >15), time from symptom onset, blood collection (dichotomized at 4.5 hours), arterial hypertension, diabetes mellitus, and atrial fibrillation. Selection of

variables that were tested for interaction was based on biological plausibility on factors that might influence the prognostic value of copeptin. NIHSS was categorized in order to represent clinically relevant stroke severity subgroups (mild, moderate, and severe strokes), and time from symptom onset was dichotomized to represent the time window for IV thrombolysis.

C-statistics. The discriminatory value of copeptin was assessed with the area under the receiver operating characteristic (ROC) curve (AUC). The incremental discriminatory value of copeptin was tested by comparing the AUC of the NIHSS (nested model), our prespecified prognostic factor of reference,[7] with the AUC of the NIHSS and copeptin (whole model), as well as comparing the logistic and Cox regression models *without* copeptin (nested models) with the same models *with* copeptin (whole models). For these comparisons of nested to whole models, we used the likelihood ratio test as recommended.[17]

Reclassification tables. For risk model of comparison, we used the multivariate models described above. As recommended in the statistical literature,[18] we calculated continuous (category-free) net reclassification improvement (NRI) values because our multivariate models of comparisons have no validated risk categories. To calculate category-based NRI values, we used the validated prognostic index by König et al.,[19] encompassing admission NIHSS score and age. Four risk categories were chosen: 0%–5%, 5%–10%, 10%–15%, and >15%.

Statistics were calculated using Stata Statistical Software: Release 12, 2011 (StataCorp LP, College Station, TX). For reclassification tables, we used R version R 2.15.1 along with the PredictABEL package (version 1.2-1) available from CRAN repository (http://cran.r-project.org/). Testing was 2-sided and p values <0.05 were considered to indicate statistical significance.

RESULTS **Study population.** From March 24, 2009 through April 8, 2011, we consecutively recruited 788 patients with ischemic stroke. Stroke treatment was conservative in 465 patients (59.4%), and 318 patients (40.6%) underwent thrombolysis. Follow-up was available in 783 patients (follow-up rate: 99.6%). The detailed patient flow is outlined in figure 1.

The median age of the cohort was 71.0 years (IQR 60.5–80.0), and 38.1% of the patients were women. The most common cardiovascular risk factor was arterial hypertension, which was present in 68.8% of patients. At admission, the median NIHSS score was 6 (IQR 3–13), and the median copeptin concentration was 14.2 pmol/L (IQR 5.9–46.5) (table 1).

Primary end points. ***Prediction of functional outcome after 3 months.*** A total of 300 patients (38.3%) had an unfavorable outcome after 3 months. Median copeptin concentration was more than 3-fold higher in patients with unfavorable outcomes than in those with favorable outcomes (table 1, figure e-1 on the *Neurology*® Web site at www.neurology.org). In the multivariate logistic regression model, higher copeptin concentrations independently predicted an unfavorable outcome (adjusted OR for any 10-fold copeptin increase 2.17 [95% CI, 1.46–3.22]). For instance, for a patient with a copeptin level of 30.0 pmol/L, the odds of unfavorable outcome are on average 2.17 times or 117% higher compared with a patient with a copeptin level of 3.0 pmol/L, after adjustment for the covariates included in the logistic regression model presented in table 2. The multivariate logistic model was well calibrated as assessed by the Hosmer and Lemeshow goodness-of-fit test ($p = 0.62$).

The discriminatory accuracy of copeptin, assessed with the area under the ROC curve, was 0.71 (95% CI, 0.67–0.75). Copeptin significantly improved the discriminatory accuracy of the NIHSS and the

Figure 1 Flowchart of patient enrollment and follow-up

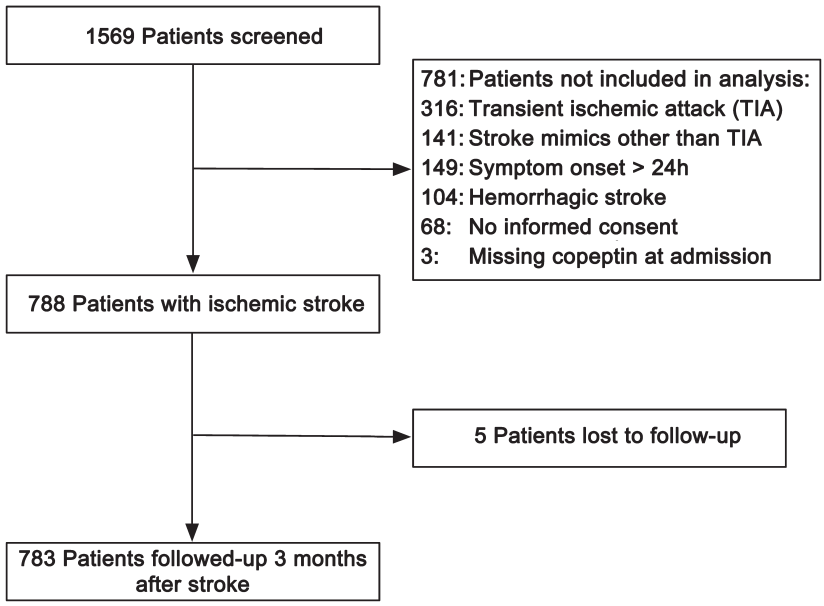


Table 1 Baseline characteristics of all patients, stratified by outcome[a]

	All patients (N = 783)	Favorable outcome (n = 483)	Unfavorable outcome (n = 300)	*p* Value
Demographic data				
Age, y, median (IQR)	71.0 (60.5–80.0)	66.2 (58.0–76.0)	77.3 (68.3–83.2)	<0.001[b]
Women, n (%)	298 (38.1)	162 (33.5)	136 (45.3)	0.001[b]
Medical history, n (%)				
Hypertension	539 (68.8)	312 (64.6)	227 (75.7)	0.001[b]
Atrial fibrillation	153 (19.5)	77 (15.9)	76 (25.3)	0.002[b]
Current smoking	138 (17.6)	98 (20.3)	40 (13.3)	0.01[b]
Diabetes mellitus	125 (16.0)	59 (12.2)	66 (22.0)	<0.001[b]
Coronary heart disease	149 (19.0)	75 (15.5)	74 (24.7)	0.002[b]
Dyslipidemia	432 (55.2)	280 (57.8)	152 (50.7)	0.001[b]
Previous cerebrovascular event	152 (19.4)	86 (17.8)	66 (22.0)	0.16
Kidney impairment[c]	174 (22.2)	82 (17.0)	92 (30.7)	<0.001[b]
Modified Charlson Index (IQR)	0 (0–1)	0 (0–1)	1 (0–2)	<0.001[b]
Clinical data, median (IQR)				
NIHSS score at admission	6 (3–13)	4 (2–7)	13 (7–18)	<0.001[b]
Body mass index, kg/m^2	25.8 (23.2–28.4)	25.9 (23.4–28.7)	25.6 (22.6–27.8)	0.14
OCSP, n (%)				
TACS	158 (20.2)	35 (7.1)	123 (41.0)	<0.001[b]
PACS	291 (37.2)	191 (39.5)	100 (33.3)	0.09
LACS	188 (24.0)	161 (33.3)	27 (9.0)	<0.001[b]
POCS	146 (18.6)	96 (19.9)	50 (16.7)	0.30
Laboratory values, median (IQR)				
Copeptin, pmol/L	14.2 (5.9–46.5)	9.6 (4.7–25.8)	32.2 (11.8–103.5)	<0.001[b]
Time to blood collection, h[d]	2.8 (1.7–5.0)	2.7 (1.7–4.6)	3.0 (1.6–5.7)	0.33
Glucose, mmol/L	6.3 (5.5–7.5)	6.0 (5.4–7.2)	6.7 (5.8–8.3)	<0.001[b]
CRP, mg/L	3.0 (3.0–6.0)	3.0 (3.0–5.0)	3.0 (3.0–9.0)	<0.001[b]
Creatinine, mmol/L	81.0 (69.0–95.0)	80.0 (69.0–92.0)	82.0 (69.0–100.0)	0.27
eGFR,[e] mL/min/1.73 m^2	75.0 (60.9–91.9)	77.6 (64.7–92.8)	70.5 (55.4–90.1)	<0.001[b]
Lesion size on MRI, DWI, n (%)[f]				
None detected	39 (7.3)	32 (8.6)	7 (4.2)	0.07
Small, 1–10 mm^3	233 (43.4)	197 (53.1)	36 (21.7)	<0.001[b]
Medium, 10–100 mm^3	205 (38.2)	128 (34.5)	77 (46.4)	0.02[b]
Large, >100 mm^3	60 (11.2)	14 (3.8)	46 (27.7)	<0.001[b]
TOAST subtype, n (%)				
Large-vessel disease	110 (14.0)	64 (13.2)	46 (15.3)	0.46
Cardioembolic	308 (39.3)	183 (37.9)	125 (41.7)	0.29
Small-artery disease	45 (5.8)	40 (8.3)	5 (1.7)	<0.001[b]
Multiple causes	70 (8.9)	52 (10.8)	18 (6.0)	0.03[b]
Other known	29 (3.7)	17 (3.5)	12 (4.0)	0.85
Undetermined	221 (28.2)	127 (26.3)	94 (31.3)	0.14

Abbreviations: CRP = C-reactive protein; DWI = diffusion-weighted imaging; eGFR = estimated glomerular filtration rate; IQR = interquartile range (meaning range between the first and third quartile); LACS = lacunar anterior circulation stroke; mRS = modified Rankin Scale; NIHSS = NIH Stroke Scale; OCSP = Oxfordshire Community Stroke Project classification; PACS = partial anterior circulation stroke; POCS = posterior circulation stroke; TACS = total anterior circulation stroke; TOAST = Trial of Org 10172 in Acute Stroke Treatment.[12]

[a] See the Statistical Analysis section for a description of this analysis. Because of rounding, percentages may not total 100.

[b] The differences between the group with a favorable outcome vs the group with an unfavorable outcome are statistically significant.

Continued

multivariate logistic regression model both for an unfavorable functional outcome, defined as mRS score 3 to 6 (table 3, figures e-2 to e-4), and disability, defined as mRS score 3 to 5 (table e-1).

Copeptin improved classification of patients when added to the validated prognostic index of König et al.[19] with a categorical NRI of 11.8% (table e-2). Among patients with a favorable outcome, a total of 11.8% were correctly moved to lower risk categories, whereas in patients with an unfavorable outcome, zero were reclassified. Moreover, adding copeptin to the full model improved reclassification as evidenced by the continuous NRI of 46.8%.

Prediction of mortality within 3 months after stroke. A total of 118 patients (15.1%) died within 3 months after stroke. The median copeptin concentration was more than 5-fold higher in patients who died within 3 months compared with survivors (58.8 pmol/L [IQR 23.0–141.0] vs 11.7 pmol/L [IQR 5.6–35.1], $p < 0.001$) (table e-3, figure e-1). Overall, Kaplan-Meier survival curves of patients stratified per copeptin tertiles differed ($p < 0.001$, log-rank test) (figure 2). In the multivariate Cox model, higher copeptin concentrations independently predicted mortality (adjusted HR for any 10-fold copeptin increase 2.40 [95% CI, 1.60–3.60], $p < 0.001$) (table 2). The multivariate Cox model was well calibrated as assessed by the Groennesby and Borgan test ($p = 0.35$). The overall discriminative ability of copeptin to distinguish survivors from nonsurvivors—assessed with the area under the ROC curve—was 0.75 (95% CI, 0.71–0.80) (figures e-5 to e-7). Copeptin significantly improved the discriminatory accuracy of the NIHSS and the multivariate Cox regression model (table 3). These results were also confirmed in reclassification statistics where the categorical NRI of copeptin on the validated prognostic index of König et al. was 37.2% (table e-4). Among survivors, 33.0% were correctly moved to lower risk categories, whereas in nonsurvivors 4.2% were correctly moved to higher risk categories. Moreover, adding copeptin to the multivariate model resulted in a continuous NRI of 64.5%.

Interaction analysis. The predictive value of copeptin regarding functional outcome and mortality was consistent across all subgroups. We did not identify any significant effect modifiers.

Secondary end point: Prediction of complications. A total of 185 patients (23.6%) had at least 1 of the 5 predefined complications during hospitalization. Median copeptin levels were more than 3-fold higher in patients developing any of the prespecified complications during hospitalization (39.9 pmol/L [IQR 16.4–114.0] vs 11.0 pmol/L [IQR 5.3–32.5], $p < 0.001$) (table e-5). In the multivariate logistic model, copeptin independently predicted the occurrence of at least 1 complication (adjusted OR for any 10-fold copeptin increase 1.93 [95% CI, 1.33–2.80], $p = 0.001$). Other significant predictors were the NIHSS score and age. Copeptin improved the prognostic accuracy of the NIHSS (AUC change from 0.79 [95% CI, 0.76–0.83] to 0.80 [95% CI, 0.77–0.84], $p = 0.001$). We found no significant interaction between copeptin and each of the potentially effect-modifying variables used for the primary outcomes. Prediction of individual complications was associated with different OR for any 10-fold copeptin increase: for symptomatic intracerebral hemorrhage, OR 1.07 (95% CI, 0.45–2.53, $p = 0.89$); for space-occupying cerebral edema, OR 2.85 (95% CI, 1.39–5.87, $p = 0.004$); for pneumonia, OR 1.79 (95% CI, 1.16–2.76, $p = 0.009$); for seizures, OR 1.20 (95% CI, 0.62–2.33, $p = 0.59$); and for mortality within 10 days from admission, OR 3.00 (95% CI, 1.67–5.39, $p < 0.001$).

DISCUSSION In this prospective, multicenter study, higher copeptin blood levels independently predicted functional outcome and mortality 3 months after ischemic stroke. Copeptin improved the discriminatory ability of the NIHSS and multivariate models as shown by an increase in the respective AUCs. Despite the modest size of the AUC increases, copeptin improved risk classification by 11.8% for functional outcome and by 37.2% for mortality compared with the validated prognostic index by König et al.,[19] encompassing NIHSS score and age. Moreover, copeptin improved reclassification compared with the multivariate models including demographic factors, cardiovascular risk factors, lesion size in MRI, comorbidities, and admission laboratory variables such as CRP and glucose. The CoRisk study confirms and extends the conclusions of the previously published derivation study.[3] Further new findings are that 1) copeptin independently predicts complications, and 2) the prognostic ability of copeptin was consistent across subgroups including different acute treatments (conservative vs thrombolysis).

[c] Kidney impairment was defined as eGFR <60 mL/min/1.73 m^2.[40]

[d] Time to blood collection was calculated in hours from symptom onset (if known).

[e] eGFR was estimated according to the Modification of Diet in Renal Disease Study.[40]

[f] Percentages refer to patients for whom information on DWI lesion was present (n = 537). Of the 537 patients undergoing an MRI on admission, 371 had a favorable outcome (69.1%) and 166 had an unfavorable outcome (30.9%) within 3 months after stroke. The definition of stroke required an acute focal neurologic deficit lasting longer than 24 hours, suggestive of acute stroke, and with no sign of acute intracranial bleeding on cerebral imaging (CT or MRI). A visible ischemic lesion on MRI was not required for the diagnosis of ischemic stroke.

Table 2 Multivariate logistic regression analysis for functional outcome and Cox regression model for mortality[a]

	Functional outcome			Mortality		
Predictors	**OR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (per y)	**1.07**	**1.04–1.09**	**<0.001**	**1.04**	**1.02–1.06**	**<0.001**
Hypertension	1.00	0.60–1.68	0.99	0.90	0.53–1.51	0.68
Diabetes mellitus	1.69	0.88–3.26	0.12	**1.78**	**1.03–3.11**	**<0.001**
Atrial fibrillation	0.69	0.39–1.22	0.20	1.14	0.66–1.96	0.64
Modified Charlson Index (per point)	1.06	0.89–1.27	0.50	**1.11**	**1.01–1.23**	**0.04**
Kidney impairment[b]	1.17	0.69–1.99	0.55	1.36	0.86–2.16	0.19
NIHSS score at admission (per point)	**1.14**	**1.09–1.21**	**<0.001**	**1.05**	**1.02–1.08**	**<0.001**
TACS	1.98	0.97–4.04	0.06	**2.02**	**1.17–3.49**	**0.01**
Log_{10} (copeptin [pmol/L])	**2.17**	**1.46–3.22**	**<0.001**	**2.40**	**1.60–3.60**	**<0.001**
Log_{10} (glucose [mmol/L])	0.55	0.07–4.50	0.58	0.68	0.10–4.66	0.69
Log_{10} (CRP [mg/L])	1.77	0.99–3.17	0.05	**1.89**	**1.20–2.96**	**0.01**
Large DWI lesion (>100 mm^3)	**4.16**	**1.62–10.65**	**0.003**	1.25	0.69–2.27	0.47
Time from symptom onset to blood collection (per h)	**1.06**	**1.01–1.11**	**0.01**	1.01	0.97–1.06	0.64
Women	1.06	0.66–1.71	0.80	NA	NA	NA
Medium DWI lesion (10–100 mm^3)	1.31	0.80–2.14	0.28	NA	NA	NA
Unclear cause of stroke	NA	NA	NA	**2.23**	**1.39–3.58**	**0.001**

Abbreviations: CI = confidence interval; CRP = C-reactive protein; DWI = diffusion-weighted imaging; eGFR = estimated glomerular filtration rate; HR = hazard ratio; NA = not applicable (meaning that the variable was not significantly associated with an unfavorable outcome or mortality in the respective univariate analysis); NIHSS = NIH Stroke Scale; OR = odds ratio; TACS = total anterior circulation stroke. Data in boldface type indicate statistically significant predictors.

[a] See the Statistical Analysis section for a description of this analysis. OR and HR refer to a 1-unit increase in the explanatory variable and to any 10-fold increase in copeptin, glucose, and CRP (log-transformed with a base of 10). For example, the odds of an unfavorable outcome are on average 2.17 times or 117% higher in a patient with a copeptin level of 30.0 pmol/L compared with a patient with a copeptin level of 3.0 pmol/L, after adjustment for the covariates included in the presented logistic regression model.

[b] Kidney impairment was defined as eGFR <60 mL/min/1.73 m^2.[40] Coronary heart disease and eGFR/creatinine were not included in the multivariate model because of collinearity with atrial fibrillation and kidney impairment, respectively.

Although several markers, such as N-terminal brain natriuretic peptide,[5,20,21] CRP,[21–24] D-dimers,[20,25,26] interleukin-6,[21,24,27] von Willebrand factor,[28,29] S-100β,[30] and neuron-specific enolase,[31] have been associated with functional outcome or mortality, only a few biomarkers added to prognosis based on clinical assessment. None of these markers, however, were fully evaluated including assessment of accuracy (i.e., calibration by goodness-of-fit test), discriminatory ability (i.e., C-statistics), reclassification improvement, and performance in an external validation study according to the recommendations of the American Heart Association for studies evaluating biomarkers in cardiovascular research.[32]

Several prognostic models have been evaluated in the past years. Some of these models include information from MRI[33] or CT[34]; others are based mostly on clinical information such as comorbidities or stroke severity.[35–37] As reference for reclassification, we chose the prognostic index of König et al.[19] because of its validation for both functional outcome and mortality at 3 months along with its robust prognostic power arising from 2 easily accessible variables such as age and NIHSS score. To be used in clinical routine, prognosis should be almost immediate and based on only a few variables, thus copeptin, which adds to the simple and highly accurate prognostic index by König et al.,[19] may be promising. Measurement of copeptin in the emergency setting (incubation time 30 minutes[6]) may help physicians to more accurately inform patients and caregivers on the overall prognosis. Copeptin might help in early decision-making on aggressiveness of care, potential new interventions, discharge planning, and rehabilitation. In the setting of trials of new stroke therapies, it might also be valuable to predict those with stroke recovery. For optimal risk stratification, it is crucial that prognostic information be available within the first hours from symptom onset, and copeptin meets this demand. The pathophysiologic mechanism relating copeptin with stroke outcome and mortality is only partially understood. Copeptin derives from a larger precursor peptide along with arginine vasopressin (AVP) and is released in an equimolar ratio to AVP. The advantage of copeptin is that it is more stable in blood circulation and easier to

Table 3 Area under the curve for selected predictors of functional outcome and mortality[a]

Predictors	ROC area	95% CI	p[b]
Functional outcome			
Copeptin, pmol/L	0.71	0.67–0.75	—
NIHSS	0.81	0.78–0.84	**<0.001**
NIHSS + copeptin, pmol/L	0.83	0.80–0.86	
Model 1	0.86	0.84–0.89	**<0.001**
Model 1 + copeptin, pmol/L	0.87	0.85–0.90	
Mortality			
Copeptin, pmol/L	0.75	0.71–0.80	
NIHSS	0.80	0.77–0.84	**<0.001**
NIHSS + copeptin, pmol/L	0.83	0.79–0.86	
Model 2	0.86	0.82–0.90	**<0.001**
Model 2 + copeptin, pmol/L	0.87	0.83–0.91	

Abbreviations: CI = confidence interval; NIHSS = NIH Stroke Scale; ROC = receiver operating characteristic.
[a] Model 1, multivariate logistic regression model presented in table 2; model 2, Cox regression model presented in table 2.
[b] To test the statistical significance of the comparisons of nested vs whole models, the likelihood ratio test was used as recommended.[17] The boldface *p* values indicate that the following differences in the ROC area are statistically significant: for functional outcome—between NIHSS and NIHSS + copeptin, between model 1 and model 1 + copeptin; for mortality—between NIHSS and NIHSS + copeptin, between model 2 and model 2 + copeptin.

measure compared with AVP.[14] The secretion of AVP can be stimulated through brainstem and limbic pathways triggered by different "stressors"; it seems to act as an endogenous barometer of integral homeostasis. Thus, copeptin assesses the severity of damage beyond lesion size, the effect of age, sex, and measurable clinical impairment on admission. In addition AVP/copeptin might be associated with adrenocorticotropic hormone–induced hypercortisolism, which is thought to potentiate ischemic neuronal injury, especially in the long run.[38] Data from experimental studies imply that AVP has a role in brain edema formation because blocking of AVP receptors attenuates brain edema in ischemic mice models.[39]

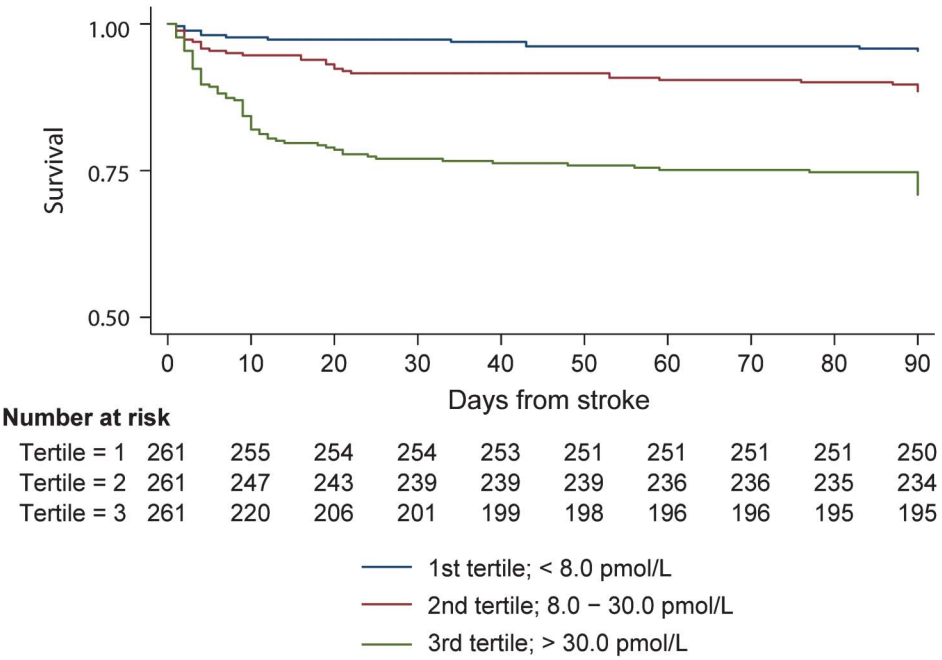
Some limitations of the study merit attention. To assess complications, a time-to-event analysis would have been ideal. However, we were not able to document the exact time of onset of some complications, e.g., aspiration pneumonia, and we did not take into account the duration of hospitalization. Moreover, this study was powered only for the combined end point of complications after stroke, but not to assess each complication separately: our subgroup analyses should be interpreted with caution, and further studies are needed to elucidate the specific association with each of the assessed complications.

In patients with ischemic stroke, copeptin is a validated blood marker that adds predictive information on functional outcome and mortality at 3 months beyond important clinical variables such as stroke severity and age. Copeptin seems to be a promising blood marker for prediction of in-hospital complications.

AUTHOR CONTRIBUTIONS

As principal investigators, Dr. De Marchis, Dr. Katan, and Dr. Arnold had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: M. Katan, G.M. De Marchis, M. Christ-Crain, B. Mueller. Acquisition of data: G.M. De Marchis, A. Weck, F. Fluri,

Figure 2 Kaplan-Meier survival estimates for patients stratified by copeptin tertiles



The numbers of patients at risk are indicated at multiples of 10 days. Overall, Kaplan-Meier survival curves of patients stratified per copeptin tertiles differed ($p < 0.001$, log-rank test).

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DISCLOSURE

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Stress hormones predict cerebrovascular re-events after transient ischemic attacks

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Stress hormones predict cerebrovascular re-events after transient ischemic attacks

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ABSTRACT

Background: TIA is a strong predictor of subsequent stroke. The hypothalamic stress hormone copeptin is an accurate prognostic marker in acute ischemic stroke. This study assessed prognostic reliability of 2 distinct stress hormones, copeptin and cortisol, for the risk stratification of re-events in patients with TIA.

Methods: We conducted a prospective study in patients admitted to the emergency department with a TIA. Clinical risk scoring using the ABCD2 score was determined and both hormones were measured in plasma on admission. The primary endpoint was a cerebrovascular re-event within 90 days.

Results: We included 107 consecutive patients with TIA. Re-events occurred in 10 patients (9%). Copeptin levels were higher in patients with a re-event compared with patients without re-event ($p = 0.02$), in contrast to cortisol ($p = 0.53$). Copeptin revealed a higher area under the receiver operating characteristics curve (AUC) to predict re-events compared to the ABCD2 score (AUC of 0.73 vs 0.43; $p < 0.01$) and improved its prognostic accuracy (AUC of combined model of 0.77; $p = 0.002$).

Conclusion: Measurement of plasma copeptin but not cortisol levels in patients with TIA provides additional prognostic information beyond the ABCD2 clinical risk score alone. If confirmed in future studies, routine copeptin measurement may be an additional tool for risk stratification and targeted resource allocation after TIA. *Neurology*® 2011;76:563–566

GLOSSARY

AUC = area under the receiver operating characteristics curve; **CI** = confidence interval; **DWI** = diffusion-weighted imaging; **HPA axis** = hypothalamic-pituitary-adrenal axis; **IQR** = interquartile range; **ROC** = receiver operating characteristic.

The risk of a stroke after a TIA in the first 90 days ranges from 9.5% to 20%.[1] The ability to accurately identify high- and low-risk patients with TIA has important clinical implications. The discriminatory ability of the 7-point ABCD2 score in patients with TIA to assess short-term risk of stroke is limited.[2] Therefore, it is important to find additional reliable and rapidly measureable predictors for recurrent vascular events in patients with TIA.

Cerebral ischemia activates the hypothalamic-pituitary-adrenal axis (HPA axis).[3] Vasopressin is a main secretagogue of the HPA axis. Copeptin is produced in equimolar amounts to vasopressin and can be easily determined.[4]

The present study evaluated copeptin and cortisol as new prognostic tools for the risk stratification of re-events in a cohort of patients with TIA.

METHODS

Standard protocol approvals, registrations, and patient consents. The design of this prospective cohort study has been described in detail (clinicaltrials.gov NCT00390962)[5] and was approved by the local ethical committee. Written informed consent was obtained from all patients.

Study design and setting. Briefly, between November 2006 and November 2007, all patients (n = 605) presenting at the emergency department of the University Hospital Basel, Switzerland, with a suspected cerebrovascular event were screened.

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The primary endpoint of this analysis was a subsequent cerebrovascular event (i.e., ischemic or hemorrhagic stroke, or TIA) in the first 90 days following index TIA, assessed by a structured follow-up telephone interview with the patient. Stroke was defined as an acute deficit of focal neurologic function with symptoms lasting more than 24 hours, resulting from intracranial vascular disturbance (ischemia or hemorrhage) occurring within 90 days after the index event. TIA was defined as an acute loss of focal cerebral or ocular function with symptoms lasting less than 24 hours of presumed ischemic origin following adequate investigations.[6]

A total of 107 patients were diagnosed with a TIA on admission. All patients with acute neurologic dysfunction, in whom diagnostic workup suggested a nonvascular disorder, were classified as TIA mimics.[6] Risk stratification according to the ABCD2 score[7] was performed. All patients underwent a standardized diagnostic workup including specification of stroke etiology according to the TOAST classification[8] and routine laboratory testing. All plasma blood samples were obtained on admission within 72 hours of symptom onset. Copeptin was measured with a new chemiluminescence sandwich immunoassay.[4] Median copeptin levels in healthy individuals are 3.7 pmol/L and the 97.5 percentile is 16.4 pmol/L.[4] Cortisol was measured with a competitive chemiluminescence immunoassay (IMMULITE 2000; Siemens Medical Solution Diagnostics, Los Angeles, CA). Cranial CT was performed on admission and thereafter MRI within 24 hours after admission on a 1.5-T MR Avanto system (Siemens, Erlangen, Germany). Diffusion-weighted imaging

Table Baseline characteristics

Characteristics	Overall	No re-events	Re-events	*p* Value
Demographic characteristics				
TIA, n (%)	107 (100)	97 (91)	10 (9)	
Death, n (%)	2 (2)	1 (1)	1 (1)	
Age, y, median (IQR)	71 (60–79)	70 (59–79)	73 (69–78)	0.26
Female, n (%)	60 (100)	45 (49)	2 (20)	0.13
TIA severity, ABCD2, median (IQR)	4 (3–5)	4 (3–5)	4(3–4)	0.50
Clinical findings, median (IQR)				
Heart rate, bpm	74 (66–84)	75 (66–85)	67 (65–69)	0.14
Arterial pressure, mm Hg				
Systolic	163 (141–182)	165 (143–184)	155 (137–176)	0.62
Diastolic	88 (81–98)	90 (82–98)	82 (73–86)	0.11
Body temperature (°C)	37.0 (36.6–37.5)	37.1 (36.8–37.5)	36.3 (36.0–36.9)	0.02
Laboratory findings, median (IQR)				
Copeptin, pmol/L	4.61 (2.83–10.14)	4.55 (2.80–7.8)	11.25 (9.11–24.78)	0.0016
Cortisol, nmol/L	408 (317–538)	408 (317–557)	382 (349–427)	0.53
C-reactive protein, mmol/L	3 (3–5.8)	3 (3–6)	3 (3–4)	0.33
Glucose level, mmol/L	5.9 (5.2–7.1)	5.9 (5.3–7.3)	6.1 (4.8–6.8)	0.58
TIA etiology, n (%)				
Small vessel occlusive	8 (7)	7 (7)	1 (10)	0.95
Large vessel occlusive	10 (9)	6 (6)	4 (40)	0.053
Cardioembolic	10 (9)	9 (9)	1 (10)	0.92
Unknown	79 (74)	75 (78)	4 (40)	0.59
Comorbidity, median (IQR)				
Charlson Index	0 (0–2)	0 (0–2)	1 (0–3)	0.38
Vascular risk factors, n (%)				
Hypertension	74 (69)	68 (70)	6 (60)	0.85
Atrial fibrillation	11 (10)	9 (9)	2 (20)	0.64
Smoking history	36 (34)	32 (33)	4 (40)	0.96
Hypercholesterolemia	35 (33)	29 (30)	6 (60)	0.63
Diabetes mellitus	16 (15)	15 (15)	1 (10)	0.76
Coronary hearth disease	19 (18)	14 (14)	5 (50)	0.25
Prior TIA or stroke	34 (32)	32 (33)	2 (20)	0.77
Family history of stroke or myocardial infarction, n (%)	39 (36)	37 (38)	2 (20)	0.60

Abbreviation: IQR = interquartile range.

(DWI) was available in 88 patients (82%). In 19 patients (17%) only cranial CT was performed (one patient had no neuroimaging against medical advice). DWI results were classified as negative or positive based on the presence or absence of an acute DWI lesion after neuroradiologic review.

Statistical analysis. Discrete variables are expressed as frequency (percentage) and continuous variables as medians and interquartile ranges (IQR). Two group comparisons were made using the Mann-Whitney *U* test. Receiver operating characteristics (ROC) were calculated. The area under the ROC curve (AUC) is a summary measure over criteria and cutpoint choices. To test whether the copeptin level improves ABCD2 score performance, we compared the nested logistic regression model with ABCD2 score and copeptin with a model limited to the ABCD2 score alone. Finally, time to re-event was analyzed in Kaplan-Meier survival curves and patients were stratified based on a priori defined median copeptin levels. Analyses were performed with STATA 9.2 (StataCorp., College Station, TX).

RESULTS

Baseline characteristics. Cerebrovascular re-events after a TIA within 90 days were observed in 10 (9%) patients; 8 (8%) out of the 10 patients presented with a new TIA, and 2 (2%) had an ischemic stroke; of these 2 patients, 1 died. Of the 97 patients without a re-event, 1 (1%) patient died.

The median ABCD2 score on admission was 4 (IQR 3–5). A >50% stenosis in a large vessel referable to the patients' symptoms was found in 10 patients (9%), and a cardioembolic source warranting anticoagulation was detected in 10 patients (9%). Small vessel disease was found in 8 patients (7%). Detailed baseline characteristics are presented in the table.

Copeptin, cortisol, and the ABCD2 score to predict re-events. Patients with a cerebrovascular re-event had higher median copeptin levels than patients without a re-event (11.25 [IQR 9.11–24.78] pmol/L vs 4.55 [IQR 2.8–7.8] pmol/L, $p = 0.016$). The median ABCD2 score was comparable in patients with and without a re-event (4 [IQR 3–4] vs 4 [IQR 3–5], $p = 0.56$).

The AUC for copeptin to predict a re-event was 0.73 (95% confidence interval [CI] 0.545–0.922). At a copeptin cutoff of 9.0 pmol/L, sensitivity was 80% with a specificity of 76% to diagnose re-event. Similarly, at a cutoff of 18 pmol/L, the respective sensitivity and specificity was 40% and 88%. Combining copeptin and the ABCD2 score in a combined logistic regression model showed an AUC of 0.77 (95% CI 0.596–0.949). This combination of the clinical score and the biomarker showed a higher overall prognostic accuracy compared with the ABCD2 score alone (0.43 [95% CI 0.291–0.570], $p = 0.002$).

Median basal cortisol levels in patients developing a cerebrovascular re-event compared to those without a re-event were similar (382.0 [IQR 331.0–437.5] nmol/L vs 408 [IQR 317–557] nmol/L, $p = 0.53$). The AUC for cortisol to predict a re-event was 0.57 (95% CI 0.438–0.697).

Copeptin and time to re-event. Patients with copeptin levels below the median value of 4.60 pmol/L had a minimal risk of cerebrovascular re-events, in contrast to patients with copeptin levels above the median value of 4.60 pmol/L, who were at higher risk to develop a new cerebrovascular event (log rank $p = 0.02$) (figure).

Copeptin and DWI. In the MRI subgroup of patients (n = 88) in whom the DWI showed ischemic cerebral lesions (n = 7) (6%), copeptin levels were higher compared to the patients without DWI lesions (12.65 [3.75–43.55] pmol/L vs 4.50 [2.70–7.57] pmol/L, $p = 0.045$).

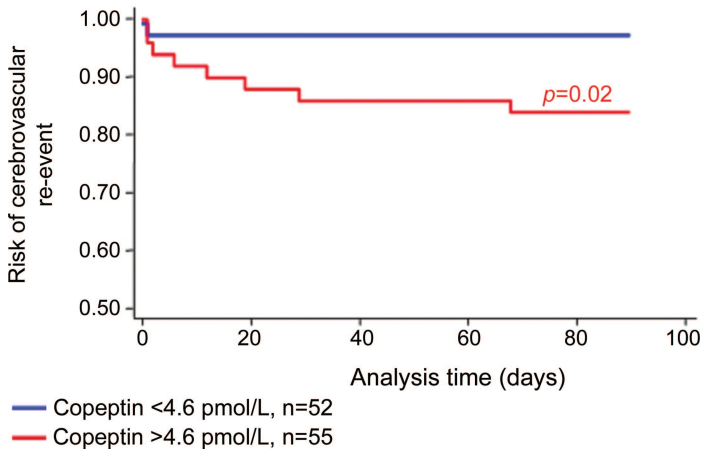
DISCUSSION

As principal finding of this study, we found that copeptin, but not cortisol levels, may provide additional prognostic information beyond the ABCD2 score in patients with a TIA to predict cerebrovascular re-events within 90 days.

Serum cortisol levels have been reported to rise proportionately with the degree of stress and to predict outcome in several diseases.[9] However, in our study cortisol levels showed no additional information for the risk stratification after a TIA. The lack of prognostic accuracy after TIA might be due to the fact that cortisol, but not copeptin, underlies a circadian rhythm and changes with food intake.[10]

Recently, we demonstrated that copeptin levels were significantly lower in healthy controls without apparent stress, compared to hospitalized patients with moderate or high stress, thereby reflecting subtle differences in individual stress level even better than cortisol.[9] This suggests that moderate stress sit-

Figure Time to re-event based on the median of copeptin



uations contribute to a notable copeptin release. We hypothesize that the activation of the stress axis in patients with a more severe "ischemic threat" (reflected by the patients who showed a DWI and/or the patients with longer lasting and more severe symptoms) might be more pronounced. These patient groups are known to have a higher risk for cerebrovascular re-events[11] and mortality. However, although copeptin in stroke patients was associated with lesion size,[5] it was still an independent predictor for functional outcome and mortality within 90 days. This suggests that copeptin not only reflects the visible DWI lesion but provides additional prognostic information by yet unknown mechanisms.

Despite the strength of our prospective enrollment and the detailed patient characterization, our study has limitations. First, the ability to accurately identify high- and low-risk patients with TIA with the ABCD2 score was very limited. It is, however, known that the discriminatory ability of the ABCD2 score especially to identify low-risk patients and to stratify patients with TIA is not optimal.[2] Second, our sample size is too small to draw definitive conclusions. A larger cohort of patients with TIA will be necessary to validate our results. Nevertheless, the results of this study should encourage and propagate further investigation in this direction.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. P. Schuetz.

DISCLOSURE

Dr. Katan has received speaker honoraria from B • R • A • H • M • S GmbH. Ms. Nigro reports no disclosures. Dr. Fluri reports no disclosures. Dr. Schuetz has received funding for travel and speaker honoraria from B • R • A • H • M • S GmbH and BioMerieux. Dr. Morgenthaler is VP Global Medical Affairs for B • R • A • H • M • S GmbH (part of Thermo-Fisher Scientific). Dr. Jax reports no disclosures. Dr. Meckel serves on a scientific advisory board for Chestnut Medical Technologies, Inc. and has received fellowship support from the Swiss Radiological Society. Dr. Gass serves on the editorial board of *Cerebrovascular Diseases*. Dr. Bingisser reports no disclosures. Dr. Steck has served on a scientific advisory board for Pfizer Inc; has received funding for travel and speaker honoraria from Talecris Biotherapeutics; and serves on the editorial boards of *Swiss Archives of Neurology and Psychiatry*, *Muscle and Nerve*, *Journal of Neurology*, *Journal of the Peripheral Nervous System*, *European Neurology*, and *Aktuelle Neurologie*. Dr. Kappos serves on the editorial board of the *International MS Journal*; receives research support from the Swiss National Research Foundation, the Swiss MS Society, and the Gianni Rubatto Foundation (Zurich); has served on scientific advisory boards and his Department at the University Hospital Basel has received research support from Acorda Therapeutics Inc., Actelion Pharmaceuticals Ltd, Abbott, AstraZeneca, Bayhill Therapeutics, Bayer Schering Pharma, Biogen Idec, Boehringer Ingelheim, Centocor Ortho Biotech Inc., Eisai Inc., Genzyme Corporation, GlaxoSmithKline, The Immune Response Corporation, MediciNova, Inc., Neurocrine Biosciences, Novartis, sanofi-aventis, Merck Serono, Roche, Teva Pharmaceutical Industries Ltd., UCB, and Wyeth. Dr. Engelter and Dr. Mueller report no disclosures. Dr. Christ-Crain has received speaker honoraria from B • R • A • H • M • S GmbH.

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Midregional Pro-Atrial Natriuretic Peptide and Outcome in Patients With Acute Ischemic Stroke

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Objectives The purpose of this study was to examine the prognostic value of midregional pro-atrial natriuretic peptide (MR-proANP) in patients with acute ischemic stroke.

Background The rapid and reliable estimation of prognosis in acute ischemic stroke is pivotal to optimize clinical care. MR-proANP, a recently described, stable fragment of the ANP precursor hormone, may be useful in this setting.

Methods In a prospective observational study, we measured MR-proANP on admission in plasma of 362 consecutive patients presenting with acute ischemic stroke. The prognostic value of MR-proANP to predict mortality within 90 days and functional outcome (defined as a modified Rankin Scale of ≤2 or ≥3) was evaluated and compared with the National Institutes of Health Stroke Scale (NIHSS) score.

Results The discriminatory accuracy, calculated with the area under the curve (AUC) of the receiver operating characteristics curve, of MR-proANP to predict death was comparable to the NIHSS (AUC: 0.86 [95% confidence interval (CI): 0.82 to 0.90] and 0.85 [95% CI: 0.81 to 0.89; $p = 0.7$]). Combined, the accuracy significantly improved (0.92 [95% CI: 0.88 to 0.96; $p < 0.01$]). The AUC of MR-proANP to predict functional outcome was 0.70 (95% CI: 0.65 to 0.75), similar to the NIHSS (0.75 [95% CI: 0.70 to 0.80]; $p = 0.16$). The prognostic value of MR-proANP for both outcomes was independent of the NIHSS. Higher MR-proANP concentrations were found in stroke of cardioembolic etiology.

Conclusions MR-proANP is a prognostic marker in the acute phase of stroke, improving the discriminatory value of the NIHSS, independently predicting post-stroke mortality and functional outcome. (The "COSMOS"-Study [Copeptin in Osmoregulation and Stress Assessment]; NCT00390962) (J Am Coll Cardiol 2010;56:1045–53) © 2010 by the American College of Cardiology Foundation

Stroke is the third leading cause of death and the primary cause of long-term disability worldwide (1). The direct and indirect cost of stroke amounted to $65.5 billion in 2008 (2). Each year, over 5 million people die as a consequence of stroke, and at least 1 in 6 patients who survives a stroke will suffer another stroke within 5 years (3). An early risk assessment with estimate of the severity of disease and prognosis could facilitate optimized care and allocation of health care resources (4). Thus, there is the need to develop a credible evidence base of prognostic information for outcomes that are meaningful to patients, including mortality and level of independency. In this context, prognostic markers available during the initial phase after acute stroke would aid in the timely estimation of disease severity, functional outcome, and mortality.

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A-type natriuretic peptide (ANP) is a family member of the natriuretic peptides. Its physiological role is mainly the regulation of blood pressure ascribed to its natriuretic,

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Abbreviations and Acronyms

AF = atrial fibrillation
ANP = A-type natriuretic peptide
AUC = area under the curve
AVP = arginine vasopressin
CE = cardioembolism
CI = confidence interval
IQR = interquartile range
LACS = lacunar syndrome
MR-proANP = midregional pro-atrial natriuretic peptide
mRS = modified Rankin Scale
NIHSS = National Institutes of Health Stroke Scale
OR = odds ratio
PACS = partial anterior circulation syndrome
POCS = posterior circulation syndrome
proBNP = pro-brain natriuretic peptide
ROC = receiver-operating characteristic curve
TACS = total anterior circulation syndrome

diuretic, and vasodilating action. ANP emerged as reliable prognostic marker for congestive heart failure and risk of cardiovascular events and death (5,6). In the acute phase of ischemic stroke, levels of ANP have been reported to be elevated (7) and to predict mortality (8). Immunohistochemical studies suggest that cerebral ischemia directly induces ANP secretion in brain tissue (9,10). Interestingly, individuals with a homozygous genotype for the ANP stop codon mutation have an increased risk of ischemic stroke (11). Thus, ANP is likely to play a role in the hemodynamic regulation during the acute phase of ischemic stroke.

Midregional pro-ANP (MR-proANP) derives from the precursor hormone of ANP. MR-proANP is released in an equimolar ratio to ANP. The present assay for MR-proANP was designed to detect the midregion of the prohormone, which is more stable than the N- or C-terminal part of the precursor (12). Other proANP assays may underestimate the release of the precursor due to an early degradation of crucial epitopes at the extreme ends of the molecule. The midregional fragment of proANP is also more stable in blood ex vivo, which renders it generally more applicable in clinical practice (13). Fragments of natriuretic prohormones (i.e., MR-proANP and pro-brain natriuretic peptide [proBNP], respectively) predict poor outcomes in patients after acute myocardial infarction and in patients with heart failure (14–18). We hypothesize that MR-proANP, measured in the acute phase after an ischemic stroke, is a good prognostic marker for functional outcome and mortality within 90 days.

Methods

Study design and setting. We conducted a prospective cohort study at the University Hospital Basel, Basel, Switzerland. From November 2006 to November 2007, consecutive patients presenting with an acute ischemic cerebrovascular event were included. The primary end point of this study was to evaluate prognostic biomarkers, particularly the arginine vasopressin (AVP) precursor (copeptin) and the precursor hormone of ANP (MR-proANP) to predict outcome in ischemic stroke. A complete description of copeptin and stroke outcome has been reported previously (19). In brief, after approval from the Ethics Committee of the University Hospital Basel was received, written informed consent was obtained from study participants or, if not feasible, from next of kin. A total of 605 consecutive patients with a suspicion of stroke within 72 h before admission at the emergency department were examined.

For the purpose of this study, we evaluated all patients with the final diagnosis of ischemic stroke (n = 362), confirmed by computed tomography and/or magnetic resonance imaging on admission. The other 243 patients had either transient ischemic attack, intracerebral hemorrhage, or other final diagnoses and were excluded from the final analysis. Neurological deficits were assessed using the National Institutes of Health Stroke Scale (NIHSS) prospectively on admission in all patients. Blood samples were collected within 0 to 3 h (n = 78), 3 to 12 h (n = 189), 12 to 24 h (n = 55), and 24 to 72 h (n = 40) from symptom onset. The clinical stroke syndrome was determined by applying the criteria of the Oxfordshire Community Stroke Project; that is, total anterior circulation syndrome (TACS), partial anterior circulation syndrome (PACS), lacunar syndrome (LACS), and posterior circulation syndrome (POCS) (20). Stroke etiology was determined according to the criteria of the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification (21), which distinguishes large artery arteriosclerosis, cardioembolism, small artery occlusion, other etiology, and undetermined etiology. At discharge from the hospital, each patient received an etiologic diagnosis of stroke.

Follow-up and end points. All patients underwent a structured telephone interview after 90 days in order to identify occurrence and timing of mortality of any cause and functional outcome. Ninety-day outcome was measured by the modified Rankin Scale (mRS), and a favorable outcome was defined as a mRS score of 0 to 2.

Assays. Blood was obtained from a venous catheter. Results of the routine blood analyses were recorded. Plasma was frozen at −70°C. MR-proANP was detected in plasma from all patients with a new sandwich immunoassay (BRAHMS AG, Hennigsdorf/Berlin, Germany), as described in detail elsewhere (13). In brief, the lower detection limit of the assay is 6.0 pmol/l. The intra-assay coefficient of variation was 10% for samples containing 23 to 3,000 pmol/l MR-proANP and 20% for samples containing 18 to 22.8 pmol/l. The interassay coefficient of variation was 10% at the concentration of 65 pmol/l MR-proANP and 20% at a concentration of 18 pmol/l MR-proANP. In 325 healthy individuals, the range of MR-proANP concentrations was 9.6 to 313 pmol/l. The median was 45 pmol/l (95% confidence interval [CI]: 43.0 to 49.1 pmol/l) (13).

Statistical analysis. Discrete variables are expressed as counts (percentage), and continuous variables as medians and interquartile ranges (IQRs). Two-group comparison of not normally distributed data was performed using Mann-Whitney *U* test and a Kruskal-Wallis 1-way analysis of

variance was used for multigroup comparisons. First, the relation of MR-proANP with outcomes (i.e., death and functional outcome in stroke patients) was assessed using logistic regression models. All baseline parameters were analyzed. Thereby, common logarithmic transformation (i.e., base 10) was performed to obtain normal distribution for skewed variables (i.e., MR-proANP concentrations) as the resulting model yielded a smaller Akaike Information Criterion, which was chosen to compare the results. We used crude models and only report odds ratios (ORs) of biological important or significant predictors in our tables. Multivariate models were adjusted for 3 biologically important outcome predictors for the end point mortality (n = 44) to avoid overfitting. For the end point functional outcome, we adjusted for all significant predictors, and then for the 4 main predictors. Note that the OR corresponds to an increase by 1 U in the explanatory variable. In terms of log-transformed MR-proANP values, the OR corresponds to a 10-fold increase.

Second, we compared the overall discriminatory ability of different predictors by calculating receiver-operating characteristic curve (ROC) analysis with the method by DeLong et al. (22). Thereby, the area under the ROC (AUC) is a summary measure over criteria and cut-point choices. The AUC summary equals the probability that the underlying classifier will score a randomly drawn positive sample higher than a randomly drawn negative sample. To test whether the MR-proANP level improves the performance of the NIHSS score, we compared ROCs of the logistic regression model combining the NIHSS score with MR-proANP with a ROC limited to the NIHSS. We also compared ROCs of MR-proANP and copeptin, which is a recently published prognostic biomarker in acute ischemic stroke.

Finally, to study the ability of MR-proANP to predict mortality, we calculated Kaplan-Meier survival curves and stratified patients by quartiles. All testing was 2-tailed, and p values <0.05 were considered to indicate statistical significance. All calculations were performed using STATA version 9.2 (Stata Corp, College Station, Texas).

Results

Baseline characteristics. Of the 362 consecutively enrolled patients with an ischemic stroke, 359 patients completed the 90-day follow-up and were analyzed; 2 patients were lost to follow-up, and 1 patient withdrew informed consent.

The median age of patients was 75 years (IQR 63 to 83 years), and 41% were women. Vital signs assessed on admission revealed a median systolic blood pressure of 160 mm Hg (IQR 140 to −180 mm Hg) and a body temperature of 37.0°C (IQR 36.5°C to 37.4°C). Regarding the neurological deficits on admission, the median NIHSS was 5 points (IQR 2 to 10 points). A total of 275 patients (77%) had a history of hypertension, 93 (26%) had hypercholesterolemia, 71 (20%) had a history of diabetes mellitus, 124 (35%) were smokers, 75 (21%) were diagnosed with atrial fibrillation (AF), 88 (25%) had a history of a previous vascular event, 91 (25%) had coronary heart disease, and 54 (15%) had a history of heart failure. According to the Oxfordshire Community Stroke Project classification, 162 (45%) had a PACS, 41 (11%) a TACS, 74 (21%) a LACS, and 83 (23%) a POCS. Baseline characteristics of the study population are provided in Table 1.

Outcome evaluation of the stroke population after 90 days showed a mortality rate of 12% (n = 44). The functional outcome assessment revealed a median mRS of 2 (IQR 1 to 4) in the whole stroke population, and 42% (n = 151) of patients had an unfavorable functional outcome defined as (mRS >2).

MR-proANP and severity of stroke on admission according to the NIHSS. MR-proANP concentrations increased with increasing severity of stroke as defined by the NIHSS (Fig. 1). MR-proANP levels in patients with a NIHSS of 0 to 6 points (n = 217) were 122.0 pmol/l (IQR 73.4 to 203.5 pmol/l), in patients with a NIHSS of 7 to 15 points (n = 90) 168.5 pmol/l (IQR 100.0 to 286.0 pmol/l), and in patients with a NIHSS >15 points (n = 55) 251.5 pmol/l (IQR 129.0 to 372.5 pmol/l) ($p < 0.0001$).

MR-proANP and death within 90 days. MR-proANP levels on admission in the 44 patients who subsequently died were about 3-fold increased as compared with survivors (345.0 pmol/l [IQR 232.0 to 465.0 pmol/l] vs. 130.5 pmol/l [IQR 78.2 to 216.5 pmol/l]; $p < 0.001$) (Fig. 2A). Univariate analysis identified MR-proANP concentrations, age, presence of TACS and POCS, history of heart failure, coronary artery disease, renal insufficiency, small vessel disease, and the NIHSS as predictors for death (Table 2). Thereby, the unadjusted OR of log-transformed MR-proANP was 128.7 (95% CI: 29.7 to 557.1), and for MR-proANP quartiles, it was 4.7 (95% CI: 2.8 to 8.0). After combining MR-proANP in bivariate logistic regression analysis with each predictor alone, it remained an independent predictor. In addition, multivariate analysis restricted to the 4 main predictors, age, the NIHSS, and TACS, MR-proANP remained an independent predictor for mortality with an adjusted OR of 61.01 (95% CI: 9.87 to 377.93); similarly after adjustment for age, the NIHSS, and heart failure, the adjusted OR was 62.83 (95% CI: 9.60 to 411.28) (Table 3). ROCs demonstrated a discriminatory accuracy (AUC) to predict mortality for MR-proANP of 0.86 (95% CI: 0.82 to 0.86), which was in the range of the NIHSS (AUC: 0.85 [95% CI: 0.78 to 0.91]) and copeptin (AUC: 0.82 [95% CI: 0.76 to 0.89]). MR-proANP had a better predictive value as compared with CRP (AUC: 0.70 [95% CI: 0.60 to 0.70]; $p < 0.05$) and glucose (AUC: 0.57 [95% CI: 0.47 to 0.66]; $p < 0.01$). The combination of MR-proANP and the NIHSS in a combined logistic model had a significantly higher discriminatory accuracy (AUC: 0.92 [95% CI: 0.88 to 0.96]) than the AUC of the NIHSS or MR-proANP alone ($p < 0.01$ and $p < 0.01$, respectively) (Fig. 3). Also,

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Table 1 Baseline Characteristics

	All	Favorable Outcome (mRS 0–2)	Unfavorable Outcome (mRS 3–6)	p Value*
n	359	208	151	
Demographic data				
Age, yrs	75 (63–83)	71 (59–80)	80 (71–86)	<0.001
Female sex	41% (149)	35% (73)	49% (76)	<0.01
Stroke severity, NIHSS	5 (2–10)	4 (2–6)	8 (4–17)	<0.001
Laboratory findings				
MR-proANP, pmol/l†	141.5 (84.1–237.8)	119.0 (72.8–187.0)	213.0 (119.0–332.0)	<0.0001
Glucose level, mmol/l	6.1 (5.5–7.4)	6.0 (5.3–7.2)	6.3 (5.6–7.7)	<0.05
C-reactive protein, mmol/l	3.6 (3.0–9.9)	3.0 (3.0–6.5)	4.90 (3.0–19.9)	<0.001
Creatinine, μmol/l	76.0 (63.0–89.0)	76.0 (63.5–89.0)	76.0 (62.5–91.0)	NS
Vital parameters on admission				
Arterial pressure, mm Hg systolic	160 (140–180)	162 (143–180)	159 (132–180)	NS
Arterial pressure mm Hg diastolic	90 (80–100)	91 (81–102)	90 (79–98)	NS
Body temperature, °C	37.0 (36.5–37.4)	37.0 (36.7–37.5)	37 (36.4–37.4)	NS
Stroke etiology‡				
Small vessel occlusive	15% (55)	18% (38)	11% (17)	NS
Large vessel occlusive	18% (65)	18% (38)	18% (27)	NS
Cardioembolic	37% (131)	36% (75)	37% (56)	NS
Other	4% (16)	5% (11)	3% (5)	NS
Unknown	26% (92)	22% (46)	30% (46)	NS
Stroke syndrome				
TACS	11% (41)	5% (11)	20% (30)	<0.0001
PACS	45% (162)	44% (92)	47% (69)	NS
LACS	21% (74)	23% (47)	18% (27)	NS
POCS	23% (83)	28% (58)	16% (24)	<0.01
Comorbidities				
Charlson Index	1 (0–2)	0 (0–2)	1 (0–2)	**<0.0001**
Hypertension	77% (275)	73% (152)	81% (123)	**<0.05**
Atrial fibrillation	21% (75)	16% (34)	27% (41)	**<0.05**
Heart failure	15% (54)	10% (21)	21% (33)	**<0.05**
Renal insufficiency	11% (39)	7% (14)	17% (25)	**<0.05**
Smoking history	35% (124)	38% (79)	30% (45)	NS
Hypercholesterolemia	26% (93)	28% (58)	23% (35)	NS
Diabetes mellitus	20% (71)	19% (39)	21% (32)	NS
Coronary heart disease	25% (91)	23% (48)	28% (43)	NS
Prior stroke	25% (88)	23% (48)	26% (40)	NS
Therapies				
Antihypertensive (prior to admission)	59% (213)	57% (119)	62% (94)	NS
ASA (prior to admission)	37% (133)	36% (74)	39% (59)	NS
Clopidogrel (prior to admission)	5% (18)	3% (7)	7% (11)	NS
Anticoagulant (prior to admission)	11% (38)	9% (18)	13% (20)	NS
Statins (prior to admission)	22% (78)	23% (47)	21% (31)	NS
Thrombolysis (on admission)	16% (59)	17% (36)	15% (23)	NS

Values are median (interquartile range) or % (n). *p values were assessed using the Mann-Whitney *U* test; †9 patients had missing values for MR-proANP; ‡some patients had 2 etiologies at the same time, and because of rounding, percentages may not sum to 1. Bold values indicate statistical significance.

ASA = acetyl salicylic acid; IQR = interquartile range; LACS = lacunar syndrome; mRS = modified Rankin Scale; NIHSS = National Institutes of Health Stroke Scale; PACS = partial anterior circulation syndrome; POCS = posterior circulation syndrome; TACS = total anterior circulation syndrome.

the combination of the MR-proANP and copeptin improved the discriminatory accuracy of copeptin alone (AUC: 0.89 [95% CI: 0.84 to 0.93]; p = 0.04). Adding copeptin to the logistic model of MR-proANP and the NIHSS did not further improve the model's discriminatory ability and revealed an AUC of 0.92 (95% CI: 0.88 to 0.96).

The time to death in the 90-day follow-up was analyzed using Kaplan-Meier survival curves based on MR-proANP quartiles. As demonstrated in Figure 4, patients in higher MR-proANP quartiles had an increase in the risk of mortality.

MR-proANP and functional outcome of stroke patients within 90 days. MR-proANP levels in patients with an unfavorable outcome were about 2-fold higher compared with patients with a favorable outcome (213.0 pmol/l [IQR 119.0 to 333.0 pmol/l] vs. 119.0 pmol/l [IQR 72.8 to 187.0

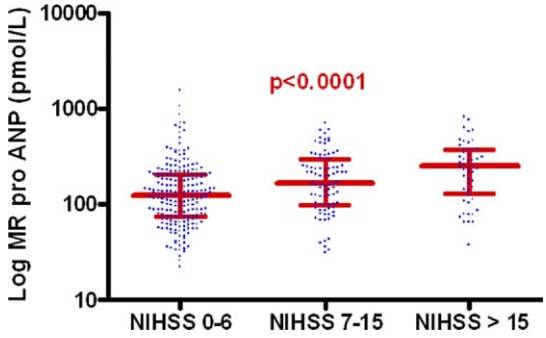

Figure 1 **MR-proANP Levels and Stroke Severity According to the NIHSS**

All data are medians and interquartile range (IQR) with dot plots representing all values. The p value refers to a Kruskal-Wallis 1-way analysis of variance. MR-proANP = midregional pro-atrial natriuretic peptide; NIHSS = National Institutes of Health Stroke Scale.

pmol/l], $p < 0.0001$) (Fig. 2B). In univariate logistic regression analysis, we calculated the predictive value of MR-proANP as compared with the NIHSS and other risk factors (Table 2). With an unadjusted OR of 10.06 (95% CI: 4.71 to 21.52), for log-transformed MR-proANP levels and 1.9 (95% CI: 1.6 to 2.4) for MR-proANP quartiles, MR-proANP was a strong predictor of outcome. Comparing MR-proANP with each significant outcome predictors in a bivariate regression model, MR-proANP remained an independent predictor (adjustment for the NIHSS revealed an OR of 5.78 [95% CI: 2.60 to 12.97; $p < 0.001$], for age an OR of 4.88 [95% CI: 2.08 to 11.46; $p < 0.001$], for female sex an OR of 9.30 [95% CI: 4.33 to 19.97; $p < 0.001$], for heart failure an OR of 9.07 [95% CI: 4.10 to 20.05; $p < 0.001$], for AF an OR of 10.08 [95% CI: 4.45 to 22.87; $p < 0.001$], for renal insufficiency an OR of 7.73 [95% CI: 3.44 to 17.35; $p < 0.001$], for CRP an OR of 6.47 [95% CI: 2.82 to 14.83; $p < 0.001$], for the Charlson Index an OR of 8.41 [95% CI: 3.84 to 18.41], and for the presence of TACS an OR of 9.34 [95% CI: 4.28 to 20.34; $p < 0.001$]). When combining all significant predictors in a multivariate model, only age, the Charlson score, and the NIHSS remained independent predictors for functional outcome.

With an AUC of 0.70 (95% CI: 0.65 to 0.75) to predict functional outcome, MR-proANP had a significantly higher prognostic discriminatory capacity as compared with TACS and sex, and was within the range of copeptin, the NIHSS, and age (Table 4). In addition, MR-proANP tended to improve the NIHSS with an AUC of the combined model of 0.79 (95% CI: 0.74 to 0.83; $p = 0.05$).

MR-proANP in different stroke etiologies. When dividing patients into 4 subgroups based on stroke etiology, 18% (n = 65) of patients were allocated to the large artery atherosclerosis group, 36% (n = 131) to the cardioembolism (CE) group, 15% (n = 55) to the small vessel occlusion group, 4% (n = 16) to the group of other etiologies (e.g., dissection), and 26% (n = 92) to the group with undetermined etiology. MR-proANP levels were highest in patients with CE etiology (206 pmol/l [IQR 119 to 326 pmol/l]), significantly higher as compared with other etiologies (124 pmol/l [IQR 73 to 207 pmol/l]; $p < 0.0001$). Logistic regression analysis revealed that MR-proANP was significantly associated with CE etiology (unadjusted OR of 7.1 [95% CI: 2.6 to 19.4]), independent of history of chronic heart failure (adjusted OR: 6.0 [95% CI: 2.3 to 13.9]) and atrial fibrillation (adjusted OR: 14.8 [95% CI: 1.2 to 6.1]) and hypertension (OR: 1.5 [95% CI: 0.80 to 2.91]). ROC analysis showed an AUC of 0.68 (95% CI: 0.62 to 0.74) for cardioembolic etiology. The optimal biomarker cutoff point for discriminating the presence or absence of a cardioembolic source was determined to be >180 pg/ml with a specificity of 60.3% (95% CI: 51.2 to 68.9), sensitivity of 71% (95% CI: 64.6 to 76.8), a positive likelihood ratio of 2.0, and a negative likelihood ratio of 0.6.

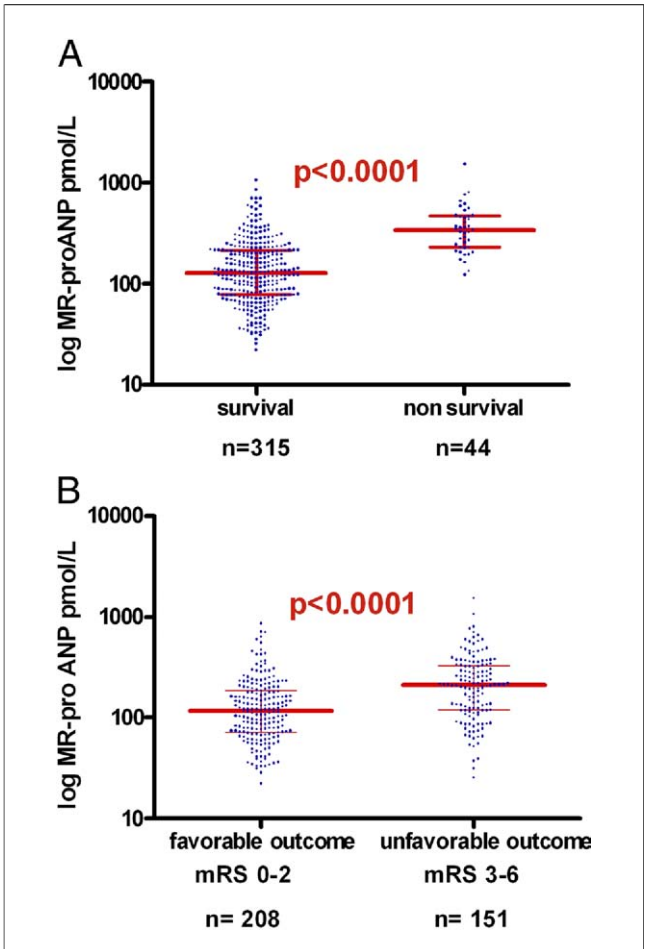

Figure 2 **Log MR-proANP Levels**

(A) Log MR-proANP levels in survivors and nonsurvivors of stroke. **(B)** Log MR-proANP levels in stroke patients with favorable and unfavorable functional outcome. All data are medians and IQR, with dot plots representing all values. The p value refers to a Mann-Whitney *U* test. Abbreviations as in Table 1.

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Table 2 Univariate Analyses

	Mortality				Functional Outcome			
	Odds Ratio	R^2	95% CI	p Value	Odds Ratio	R^2	95% CI	p Value
Laboratory parameters								
MR-proANP*	128.31	0.25	29.71–554.22	**<0.0001**	10.07	0.09	4.71–21.52	**<0.0001**
C-reactive protein	1.10	0.03	0.99–1.21	0.05	1.01	0.02	1.00–1.02	0.01
Creatinine	1.00	0.0006	0.99–1.01	0.68	1.00	0.0006	0.99–1.00	0.59
Demographic data								
Age	1.09	0.12	1.05–1.14	**<0.0001**	1.06	0.09	1.04–1.08	**<0.0001**
Female sex	1.35	0.003	0.72–2.54	0.93	1.78	0.01	1.17–2.74	**0.01**
Stroke severity, NIHSS	1.20	0.28	1.14–1.25	**<0.0001**	1.16	0.15	1.12–1.21	**<0.0001**
Comorbidities								
Charlson Index	1.16	0.008	0.96–1.40	0.13	1.34	0.03	1.15–1.56	**<0.0001**
Heart failure	2.44	0.02	1.16–5.10	**0.02**	2.49	0.003	1.38–4.51	**0.003**
Atrial fibrillation	3.16	0.001	0.92–6.15	0.09	1.91	0.01	1.14–3.19	**0.01**
Coronary heart disease	2.07	0.017	1.07–4.00	**0.03**	1.33	0.003	0.82–2.14	0.25
Renal insufficiency	3.64	0.046	1.65–8.04	**0.001**	2.92	0.02	1.46–5.83	**0.002**
Stroke syndrome								
TACS	4.44	0.10	2.15–9.19	**<0.0001**	6.67	0.05	3.39–13.99	**<0.0001**
PACS	1.12	0.0001	0.74–1.69	0.74	0.81	0.0002	0.41–1.54	0.53
LACS	0.75	0.02	0.44–1.26	0.28	0.35	0.0001	0.12–1.02	0.54
POCS	0.49	0.006	0.29–0.83	**0.01**	0.50	0.01	0.20–1.23	0.20
Stroke etiology								
Small vessel occlusive	0.11	0.03	0.02–0.84	**0.03**	0.58	0.007	0.30–1.05	0.07
Large vessel occlusive	0.55	0.006	0.21–1.45	0.23	0.97	0.00001	0.56–1.68	0.93
Cardioembolic	1.54	0.007	0.82–2.92	0.43	1.05	0.0001	0.68–1.62	0.84
Other	0.47	0.002	0.06–3.64	0.46	0.61	0.001	0.21–1.80	0.37
Unknown	1.96	0.01	1.02–3.79	0.05	1.54	0.007	0.96–2.49	0.08

p values in **bold** indicate statistical significance. *Note that the odds ratio corresponds to a unit increase in the explanatory variable; for MR-proANP this corresponds to an increase per unit of the log transformation of MR-proANP (thus a log-transformed increase of 1 corresponds to a MR-proANP increase of 10 pmol/l).Bold values indicate statistical significance.

To estimate whether MR-proANP improved the diagnosis of CE etiology by already known clinical information, we calculated logistic models based on clinical information (age, known heart failure, and known AF on admission) and combined models based on clinical information plus MR-proANP concentrations. The model including MR-proANP (AUC: 0.81 [95% CI: 0.77 to 0.86]) was significantly better than the model based on clinical information alone (AUC: 0.76 [95% CI: 0.71 to 0.81]; $p < 0.001$).

MR-proANP levels in the subgroup of patients with heart failure or AF. In 54 (15%) patients with known chronic heart failure on admission, MR-proANP levels were higher as compared with patients without chronic heart failure (271 pmol/l [IQR 162.5 to 383.8] vs. 132.0 pmol/l (IQR 78.1 to 216.6]; $p < 0.001$). MR-proANP remained a significant predictor for both mortality and functional outcome after adjustment for heart failure with ORs of 148.83 (95% CI: 32.16 to 688.67) and 9.07 (95% CI: 4.10 to 20.05), respectively. In addition, ROC analysis showed a similar predictive value of MR-proANP for both

Table 3 Multivariate Analysis for Mortality

Predictors	Odds Ratio	95% CI	p Value
MR-proANP	62.83	9.60–411.28	**<0.001**
Age	1.04	0.99–1.09	*0.07*
Stroke severity, NIHSS	1.19	1.12–1.26	**<0.001**
Chronic heart failure	0.76	0.26–2.23	*0.60*

p values in **bold** indicate statistical significance; p values in *italics* indicate nonsignificance. Abbreviations as in Tables 1 and 2.

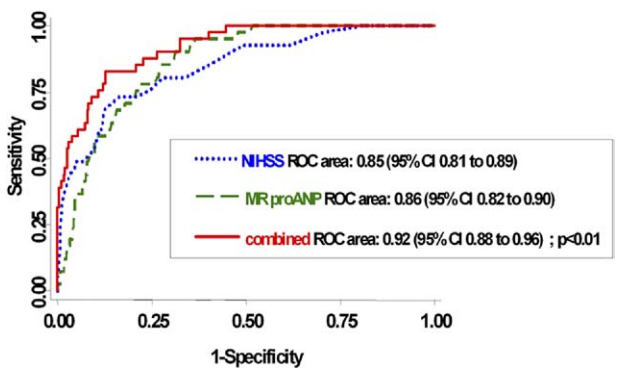

Figure 3 ROC Area to Predict Mortality

Area under the receiver-operating characteristics curve (ROC) of the NIHSS and MR-proANP, and the combined AUC. The combined model (AUC of the NIHSS and AUC of MR-proANP) was more accurate to discriminate survivors from non-survivors as compared with the clinical score (NIHSS) or the biomarker (MR-proANP) alone ($p < 0.01$ and $p < 0.01$, respectively). Abbreviations as in Figure 1.

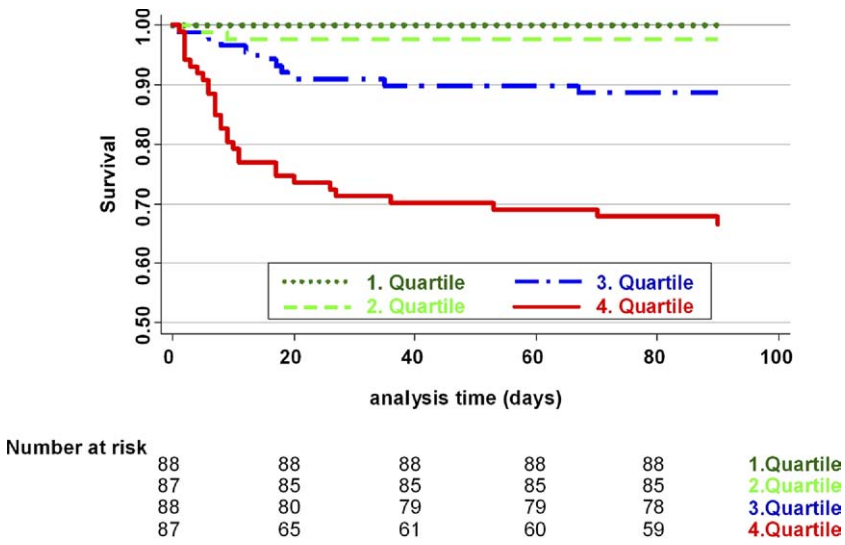

Figure 4 Kaplan-Meier Survival Curves

Estimates by midregional pro-atrial natriuretic peptide (MR-proANP) quartiles and numbers at risk. Note that in 9 patients, we had missing values for MR-proANP.

outcomes in patients with and without heart failure (data not shown).

Similarly, in 75 (21%) patients with AF, MR proANP levels were higher (258.5 pmol/l [IQR 181.8 to 380.0 pmol/l]) as compared with patients without AF (123.5 pmol/l [IQR 74.2 to 213.0 pmol/l]; $p < 0.001$). Again, MR-proANP remained a significant predictor for both outcomes after adjustment for AF. ROC analysis showed a similar predictive value of MR-proANP for both outcomes in patients with and without AF (data not shown).

Discussion

MR-proANP levels on admission were increased in stroke patients, with particularly prominently elevated levels in patients with cardioembolic strokes. MR-proANP proved to accurately predict 90-day mortality and functional outcome, independent of other comorbidities such as chronic heart failure and AF, age, and clinical scores. Importantly, MR-proANP improved the prognostic value of the NIHSS. To our knowledge, this is the first prospective study evaluating MR-proANP levels in a large cohort of stroke patients.

Table 4 ROC Analysis of Functional Outcome

Parameter	AUC	95% CI	p Value*
MR-proANP	0.71	0.65–0.75	
Copeptin	0.73	0.67–0.78	0.62
NIHSS	0.75	0.70–0.80	0.21
Age	0.70	0.65–0.75	0.88
Charlson score	0.63	0.58–0.69	0.06
Sex	0.58	0.52–0.63	**0.002**
Combined score (Copeptin and MR-proANP)	0.73	0.68–0.80	0.78
Combined score (NIHSS and MR-proANP)	0.79	0.74–0.83	**0.016**

*p values indicate significance of area under the curve (AUC) between predictors and MR-proANP. p values in **bold** indicate statistical significance.

ROC = receiver-operating characteristic curve; other abbreviations as in Tables 1 and 2.

Elevated concentrations of natriuretic peptides, especially BNP, have been shown to be of prognostic value in patients with congestive heart failure and myocardial ischemia (assessed by electrocardiographic changes) (18,23,24). Recently, MR-proANP levels were also evaluated in patients with heart failure showing comparable prognostic capabilities to BNP values with respect to 1-year all-cause mortality (25). Hence, higher natriuretic peptide levels reflect a greater degree of hemodynamic dysfunction and explain the increased mortality in patients with acute cardiac disease. A role of natriuretic peptides in the hemodynamic regulation has also been shown during the acute phase of ischemic stroke (7,26–28). Clinical studies have revealed elevated levels of natriuretic peptides in the acute phase of ischemic stroke (8,28–30). One study (8) reported elevated levels of BNP and ANP in 51 ischemic stroke patients compared with healthy controls and higher levels of natriuretic peptides in patients who died as compared with those who survived. BNP and its N-terminal peptide (NT-proBNP) has been demonstrated to be excellent markers for vascular mortality and re-events in stroke (6) although NT-proBNP levels on admission were not significantly associated with functional outcome within 3 months (30) if adjusted for vascular risk factors in another study. Estrada et al. (7) investigated ANP concentrations in 37 patients with acute ischemic stroke. Compared with healthy controls, concentration of ANP increased immediately after stroke and remained elevated for 7 days. We

found no published data on ANP or its precursor protein on the independent predictive value concerning mortality as well as functional outcome after an acute ischemic stroke.

The relation of natriuretic peptides and stroke prognosis seems not to be monocausal and is complex. First, it has been demonstrated that a higher level of NT-proBNP in stroke patients is associated with increased sympathetic activation. Sympathetic activation by itself is a prognostic determinant after an acute thromboembolic stroke (31,32). Second, previous studies have shown that heart failure is associated with dependency after stroke (27) and that it is an independent predictor for mortality after first cerebral infarction (33–35).

In our study, unadjusted and adjusted logistic regression analysis revealed that levels of MR-proANP were predictors for 90-day mortality and functional outcome, independently of the presence of heart failure or AF. The pathophysiological mechanism explaining this independent predictive value of MR-proANP observed in our study remains to be clarified. It may be hypothesized that high MR-proANP concentrations indicate the presence of a profound neurohormonal dysfunction, and thus a worse outcome. Furthermore, increased MR-proANP levels might mirror, not only manifest heart failure, but also a beginning cardiac pathology (e.g., subclinical heart failure) in which intracardial thrombus development might be more likely. This would explain the additional diagnostic value of MR-proANP levels to differentiate CE etiologies from others. It is essential to identify the CE etiology in stroke because recurrent stroke occurs within 2 weeks in up to 12% of patients who initially experience embolic stroke from cardiac sources (36). AF might be no longer present when patients are examined by 24-h electrocardiography monitoring. Thus, anticoagulant therapy might be delayed even when the neurologist suspects an embolic origin due to distinct patterns of lesions. Using biomarkers may be a reasonable strategy to improve the identification of cardioembolic stroke already in the acute phase, thus rapidly point to need of other diagnostic tests and accelerating the start of optimal secondary prevention (26).

Other known mediators involved in the ischemic cascade are markers for neuronal cell death, such as protein S-100β (37), inflammation markers, such as interleukin-6 (38), or markers for oxidative stress and blood-brain barrier disruption, such as matrix metalloproteinase-9 (39,40). Although these biomarkers reliably mirror the initial stroke severity (NIHSS and lesion size) and some even bear an association with outcome, they were not able to independently predict functional outcome and death within 3 months as well as to improve the NIHSS. To our knowledge, only 1 circulation biomarker, copeptin, the c-terminal part of the AVP prohormone, was able to significantly improve prediction of clinical outcome after stroke for death and functional outcome (19). Copeptin is released in an equimolar ratio to AVP and is more stable in the circulation and easier to determine as AVP (41). Interestingly, MR-proANP seems to be a better predictor for mortality, whereas copeptin had a higher prognostic accuracy to predict functional outcome. One reason for the absence of a "unique" biomarker preeminently mirroring all aspects of prognosis might be that the complexity of brain ischemia and recovery capacities is less amenable to the use of a single biochemical marker. In the difficult task of outcome prediction, it seems, therefore, reasonable to rely on several parameters, each mirroring different pathophysiological aspects. The approach of using a multiple biomarker panel has already been well established in the clinical setting of patients with cardiovascular disease (6,42). A model combining biomarkers and clinical parameters might also be the most promising approach to predict outcome in stroke patients.

Study limitations. We are aware of the following limitations. First, our study is a single-center study and should be externally validated in a large cohort of patients. Second, we did not directly compare the prognostic value of MR-proANP with other natriuretic peptides, especially B-type natriuretic peptides. However, this study shows promising data for a further multicenter trial including MR-proANP in a prognostic biomarker panel.

Conclusions

The present study shows that MR-proANP is a new, valuable marker for the prognosis in patients with ischemic stroke. It also seems to act as a diagnostic marker by differentiating patients with a CE etiology of stroke from other etiologies, especially when combining it with clinical information. Early risk assessment in acute ischemic stroke may allow for better and earlier intervention and improved care strategies to effectively change the dismal natural history of stroke.

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Key Words: biomarker ■ MR-proANP ■ outcome ■ stroke.

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Copeptin, Procalcitonin and Routine Inflammatory Markers–Predictors of Infection after Stroke

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Abstract

Background: Early predictors for the development of stroke-associated infection may identify patients at high risk and reduce post-stroke infection and mortality.

Methods: In 383 prospectively enrolled acute stroke patients we assessed time point and type of post-stroke infections (i.e. pneumonia, urinary tract infection (UTI) other infection (OI)). Blood samples were collected on admission, and days 1, and 3 to assess white blood cells (WBC), monocytes, C-reactive protein (CRP), procalcitonin (PCT), and copeptin. To determine the magnitude of association with the development of infections, odds ratios (OR) were calculated for each prognostic blood marker. The discriminatory ability of different predictors was assessed, by calculating area under the receiver operating characteristic curves (AUC). Prognostic models including the three parameters with the best performance were identified.

Results: Of 383 patients, 66 (17.2%) developed an infection after onset of stroke. WBC, CRP, copeptin and PCT were all independent predictors of any infection, pneumonia and UTI developed at least 24 hours after measurements. The combination of the biomarkers WBC, CRP and copeptin (AUC: 0.92) and WBC, CRP and PCT (AUC: 0.90) showed a better predictive accuracy concerning the development of pneumonia during hospitalization compared to each marker by itself (p-Wald <0.0001).

Conclusion: Among ischemic stroke patients, copeptin, PCT, WBC and CRP measured on admission were predictors of infection in general, and specifically for pneumonia and UTI within 5 days after stroke. The combination of these biomarkers improved the prediction of patients who developed an infection.

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Introduction

Infection during the first days after ischemic stroke (IS) occurs in 25–65% of patients [1,2]. Pneumonia and urinary tract infection (UTI) are the most common infectious complications after IS [3]. It has been suggested that the predominance of infections during the acute phase of stroke [1] is due to stroke-induced immunosuppression (SIS) [4]. The central nervous system modulates the activity of the immune system through complex pathways that include the hypothalamic pituitary adrenal axis (HPAA), the vagus nerve, and the sympathetic nervous system [5,6]. Several studies found an independent association between stroke-associated infections (SAI) and poor functional outcome after IS [7–9].

Therefore, early initiation of antibiotic treatments is recommended if infection is present [10]. However, gold-standard clinical diagnostics are time-consuming and delay early antibiotic therapy. Thus, accurate and simply available prognostic markers for optimal risk stratification are needed. We therefore selected C-reactive protein (CRP), white blood cells (WBC), monocytes (Mcyt), as they represent the most commonly measured and well-established inflammatory markers in clinical routine. Procalcitonin (PCT) was selected to better discriminate infections from general inflammation [11,12]. Copeptin, a reliable stress marker [13] was selected because SIS may be mediated by changes in the neuroendocrine system. All these biomarkers are available immediately due to rapid analytic procedure.

We hypothesize that these blood markers are predictive for the development of post-stroke infections. First we planned to evaluate the prognostic value of each blood biomarker to predict infections in the acute phase of IS. Second, we aimed to identify the best prognostic model consisting of a batch of the best prognostic biomarkers. Thereafter, the prognostic value of this batch was compared to that of each prognostic biomarker alone.

Patients and Methods

Ethics Statement

The study has been approved by the local Ethics Committee at the University Hospital of Basel. All participants or their representative gave written informed consent for the study.

Study Population

We performed a post-hoc analysis of a prospective cohort study [14]. All patients with IS within 72 hours before admission at the Emergency Department, University Hospital of Basel, were eligible and prospectively enrolled (11/2006–11/2007). IS was confirmed by CT and/or MRI on admission. Neurological deficits were measured at presentation with the National Institutes of Health Stroke Scale (NIHSS) score.

Definition of Stroke-associated Infections

SAI was defined as any infection occurring within the first 5 days of hospital admission [13]. Infections were diagnosed according to the criteria of the U.S. Centers for Disease Control and Prevention (CDC) [15]. We distinguished between pneumonia, urinary tract infection (UIT) and "other infections" (OI). Pneumonia was diagnosed when at least one of each of the first and latter criteria was fulfilled: i) abnormal respiratory examination, pulmonary infiltrates in chest x-rays; ii) productive cough with purulent sputum, positive microbiological cultures from lower respiratory tract or blood cultures. Diagnosis of UTI was based on two of the following criteria: fever ($\geq$38.0°C), urine sample positive for nitrite, leukocyturia (>40/μL), or significant bacteriuria ($\geq 10^4$/mL of an uropathogen). OI was defined if temperature was $\geq$38.0°C, white blood cell count was $\geq$11000/mL or CRP$\geq$10 mg/L and an infectious manifestation was present. Diagnosis of infection was done by the treating physician during hospitalization and was then validated post-hoc using charts, both diagnosis by treating physicians as well as secondary validation was blinded to biomarker levels with the exception of WBC and CRP for the diagnosis of (OI). Time point of diagnosis was referred to the beginning of clinical symptoms, which lead to diagnostic work-up and resulted in the diagnosis of infection.

In order to exclude acute infections preceding stroke, patients with admission temperature $\geq$38°C, or patients reporting an infection lasting up to 3 days before onset of stroke or patients who required mechanical intubation were not included in the study.

Laboratory Methods

Blood samples were collected on admission (baseline) within 72 hours from symptom onset, and 1, and 3 days after admission to assess WBC and Mcyt count, CRP level, PCT and copeptin. PCT serum concentration was measured using a commercially available time-resolved amplified cryptate emission technology assay (Kryptor PCT, Brahms, Hennigsdorf, Germany) [16]. Measurement of copeptin was performed in a single batch with a commercial sandwich immunoluminometric assay (LUMItest CT-proAVP, B.R.A.H.M.S, Hennigsdorf/Berlin, Germany) [17]. In patients who died within 5 days after admission, or in patients who were discharged before day 5, only data from admission or until the day of discharge were collected.

Statistical Analysis

Descriptive statistics were expressed as means ± standard deviations, medians and quartiles or absolute and relative frequencies depending on their distribution. Group differences were assessed using the Kruskal-Wallis test or Chi 2-test. Logarithmic transformation was performed to obtain an approximately normal distribution for all parameters except temperature and Mcyt.

First, the association of the biomarkers measured at admission with the presences of infections developed within 5 days was assessed using simple logistic regression.

Second we calculated pooled logistic regression considering patients to be at risk until the manifestation of an infection or until day 5 whichever occurred first. Each of these models had one time dependent predictor variable, i.e., the measurement of a given blood parameter 1 or 2 days before the respective day of diagnosis of infection. To adjust for potential clustering of data within subjects, robust standard errors were computed using the method of Huber-White. Odds ratios (OR) and associated 95% confidence intervals (95%CI) refer to an increase of the respective parameter from the lowest to the highest quartile.

Third, we compared the discriminatory ability of different predictors by calculating receiver operating characteristic (ROC) analysis. Bootstrap methods were used to derive 95%CIs for AUCs, index of Youden and optimal cutpoints to statistically compare AUC's of different predictors.

Forth, to assess the prognostic independence from age, NIHSS score (as indicator of stroke severity) and Charlson index (as indicator of comorbidity burden) as well as infratentorial and supratentorial infarct localization, we performed bivariate logistic regression (to avoid over-fitting) with these potential confounders.

Finally, we calculated 2 prediction models (batch 1 and 2) by including established inflammatory parameters (WBC and CRP) and either Copeptin or PCT, the 2 new makers. Since robust precision estimates were used, model comparisons could not be done using likelihood ratio tests but were based on Wald p-values.

P-values less than 0.05 were considered to indicate statistical significance. All calculations were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline Data

Of 383 patients with stroke, 66 (17.2%) developed an infection within 5 days after onset of stroke. Twenty (5.2%) patients suffered from pneumonia, 25 (6.5%) patients had UTI and 21 (5.5%) patients an OI (sepsis: 7 patients; phlebitis: 6 patients; gastroenteritis: 4 patients, erysipelas: 1 patient; panniculitis: 1 patient, colpitis: 2 patients). Baseline data are summarized in table 1.

Blood Biomarkers as Predictors of Post-stroke Infections

Copeptin, PCT, WBC and CRP-levels on admission predicted any infection, pneumonia and UTI in the acute phase of stroke. ORs and AUCs for each marker measured on admission (i.e. day 0) are provided in table 2. ORs to predict infections associated with nearest predictor measurements over time (i.e. performed 1 or 2 days prior to the onset of infection) are presented in table 3. After adjusting for either age, NIHSS, CI or infarct localization (infra−/supratentorial) in a bivariate model all biomarkers remained significant predictors (table 4).

Copeptin as a new prognostic marker for SAI was a strong predictor of any infection, pneumonia and UTI (table 3). Copeptin had the same prognostic accuracy compared to WBC, CRP, and the only statistical significant difference in AUCs was found when comparing WBC and copeptin regarding the outcome of OI ($p = 0.02$) (table 5).

Predictive Models for Post-stroke Infections

We defined two batches of the three parameters with highest AUC values for any infection, pneumonia, UTI and OI by

Table 1. Baseline Data.

	All patients	Patients without infection	Patients with any infection	Pneumonia	UTI	Other infections
N	383	317	66	20	25	21
Age						
Median (±SD)	71.4±13.7	70.5±14.1	75.6±10.6	77.0±10.5	77.3±10.8	74.4
Gender (male)						
% (n)	57.7 (221)	60.8 (192)	43.3 (29)	45.0 (9)	32.0 (8)	50 (13)
Laboratory Findings on admission						
CRP (mg/ml)						
median	3.0	3.0	5.1	5.6	4.9	4.5
(IQR)	(3.0–6.7)	(3.0–5.8)	(3.0–15.8)	(3.0–19.7)	(3.0–24.3)	(3.0–8.8)
WBC (10^9/l)						
median	8.0	7.8	9.7	9.8	9.9	9.2
(IQR)	(6.6–9.8)	(6.5–9.4)	(7.5–11.4)	(7.5–13.5)	(8.3–11.2)	(7.4–11.3)
Monocyte (10^9/l)						
Mean (±SD)	0.410±0.167	0.398±0.143	0.463±0.243	0.557±0.357	0.471±0.277	0.413±0.152
Procalcitonin (μg/l)						
median	0.017	0.016	0.018	0.022	0.017	0.027
(IQR)	(0.01–0.02)	(0.01–0.02)	(0.01–0.03)	(0.02–0.03)	(0.01–0.04)	(0.01–0.03)
Copeptin (pmol/l)						
median	8.19	7.68	19.6	24.1	24.5	15.0
(IQR)	(4.4–31.4)	(4.2–16.5)	(6.2–61.9)	(8.6–42.4)	(5.2–73.5)	(5.7–62.3)
Temperature (°C)						
Mean (±SD)	37.0±0.6	37.0±0.6	36.9±0.7	37.0±0.9	36.8±0.7	37.0±0.7
Risk factors % (n)						
Heart failure	13.4 (48/357)	11.6 (34/293)	21.9 (14/64)	25.0 (5/20)	17.4 (4/23)	20.0 (5/25)
AH	80.0 (286/358)	77.7 (227/292)	89.4 (59/66)	85.0 (17/20)	91.7 (22/24)	88.5 (23/26)
PAD	8.3 (30/363)	8.4 (25/298)	7.7 (5/65)	10.0 (2/20)	4.3 (1/23)	7.7 (2/26)
Diabetes mellitus	19.3 (71/367)	18.9 (57/301)	21.2 (14/66)	35.0 (7/20)	25.0 (6/24)	7.7 (2/26)
CHD	21.0 (76/363)	21.2 (63/297)	19.7 (13/66)	25.0 (5/20)	16.7 (4/24)	19.2 (5/26)
Atrial fibrillation	19.4 (69/355)	15.9 (46/289)	34.8 (23/66)	45.0 (9/20)	25.0 (6/24)	38.5 (10/26)
Hyperchol	29.2 (99/339)	29.1 (82/282)	29.8 (17/57)	41.2 (7/17)	25.0 (5/20)	21.7 (5/23)
Family history of stroke	30.1 (106/352)	31.3 (90/288)	25.0 (16/64)	25.0 (5/20)	24.0 (6/25)	21.7 (5/23)
NIHSS						
Median	5	4	11	12	9	11
(IQR)	(2–10)	(2–7)	(5–18)	(5–19)	(3–15.5)	(5.5–19)
Charlson Index						
Median	1	0	1	1.5	1	0.5
(IQR)	(0–2)	(0–2)	(0–2)	(0–2.5)	(0–2)	(0–2)
BP on admission						
Systolic BP						
Mean (±SD)	160±29	161±34	158±34	153±36	158±36	159±34
Diastolic BP						
Mean (±SD)	86±21	85±20	92±23	103±30	89±22	92±18

UTI: urinary tract infection; CRP: C-reactive protein; WBC: white blood cells; NIHSS: National Institutes of Health Stroke Scale; BP: blood pressure; IQR: interquartile range (log transformed), AH: arterial hypertension; PAD: peripheral artery disease; CHD: coronary heart disease; Hyperchol: Hypercholestrolemia.

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combining WBC, CRP and copeptin (batch 1) as well as WBC, CRP and PCT (batch 2).

Batch 1 (WBC, CRP, copeptin) better predicted any infection (Wald-p<0.001) and pneumonia (Wald-p<0.001) than the best single predictor alone. However, batch 1 was not a better predictor of UTI (Wald-p = 0.058) and OI (Wald-p = 0.25) than WBC (table 6).

Table 2. OR/AUC to predict infections (measurements on admission (day 0)).

Univariate analyses variables	Odds Ratio	CI (95%)	p-value	AUC
Any Infection (n = 66)				
Temperature	0.88	0.59–1.33	0.055	0.51
PCT	1.91	1.38–2.63	<.001	0.68
CRP	1.50	1.22–1.84	<.001	0.65
WBC	3.35	2.14–5.23	<.001	0.74
Mcyt	1.43	1.03–2.00	0.035	0.56
Copeptin	2.51	1.68–3.75	<.001	0.73
Pneumonia (n = 20)				
Temperature	0.90	0.48–1.69	0.75	0.49
PCT	1.96	1.34–2.86	<.001	0.69
CRP	1.67	1.25–2.24	<.001	0.77
WBC	3.38	1.85–6.20	<.001	0.76
Mcyt	2.00	1.28–3.11	0.002	0.63
Copeptin	2.35	1.29–4.28	0.005	0.75
Urinary Tract Infection (n = 25)				
Temperature	0.77	0.40–1.48	0.43	0.56
PCT	1.90	1.30–2.78	<.001	0.70
CRP	1.61	1.20–2.16	0.002	0.65
WBC	3.23	1.75–5.96	<.001	0.77
Mcyt	1.46	0.89–2.40	0.14	0.54
Copeptin	2.99	1.60–5.60	<.001	0.77
Other Infection (n = 21)				
Temperature	0.99	0.48–2.04	0.97	0.46
PCT	1.48	0.96–2.28	0.08	0.66
CRP	1.36	0.96–1.91	0.08	0.60
WBC	4.14	2.13–8.02	<.001	0.78
Mcyt	1.72	1.07–2.76	0.02	0.71
Copeptin	1.70	0.86–3.37	0.13	0.67

OR referred to an increment to predict values from the 1st to the 3th interquartile range (IQR). IQRs for the parameters are given in Table 1. PCT: procalcitonin; CRP: C-reactive protein; WBC: white blood cells; Mcyt: monocytes.
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Table 3. Odds ratios/AUC to predict infections associated with nearest predictor measurements*.

Univariate analyses variables	Odds Ratio	CI (95%)	p-value	AUC
Any Infection				
Temperature	2.30	1.46–3.63	0.0003	0.64
PCT	1.69	1.30–2.20	<.0001	0.67
CRP	2.28	1.75–2.96	<.0001	0.74
WBC	4.91	3.38–7.14	<.0001	0.82
Mcyt	1.72	1.40–2.11	<.0001	0.65
Copeptin	2.40	1.81–3.20	<.0001	0.75
Pneumonia				
Temperature	3.00	1.22–7.37	0.02	0.67
PCT	1.95	1.36–2.79	0.0003	0.71
CRP	2.65	1.83–3.84	<.0001	0.80
WBC	4.29	2.52–7.31	<.0001	0.81
Mcyt	2.17	1.71–2.77	<.0001	0.72
Copeptin	3.32	2.32–4.76	<.0001	0.86
Urinary Tract Infection				
Temperature	1.64	0.77–3.48	0.20	0.57
PCT	1.61	1.18–2.20	0.003	0.63
CRP	2.26	1.52–3.37	<.0001	0.74
WBC	4.65	2.85–7.58	<.0001	0.83
Mcyt	2.04	1.60–2.60	<.0001	0.69
Copeptin	2.09	1.39–3.13	0.0004	0.71
Other Infection				
Temperature	6.82	2.34–19.89	0.0004	0.80
PCT	1.33	0.95–1.85	0.09	0.58
CRP	2.31	1.50–3.55	0.0001	0.74
WBC	5.69	3.44–9.39	<.0001	0.84
Mcyt	1.32	0.93–1.87	0.12	0.61
Copeptin	2.22	1.39–3.55	0.0008	0.75

OR referred to an increment to predict values from the 1st to the 3th interquartile range (IQR). IQRs for the parameters are given in Table 1. PCT: procalcitonin; CRP: C-reactive protein; WBC: white blood cells; Mcyt: monocytes performed 1 or 2 days prior to the onset of infection.
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Batch 2 (WBC, CRP, PCT) better predicted any infection (Wald-p<0.001), pneumonia (Wald-p<0.001) and UTI (Wald-p = 0.014) than the best single predictor alone. However, batch 2 was not better in predicting OI compared to the best single predictor (Wald-p = 0.25) (table 6).

Discussion

The value of rapidly available blood markers as predictors for SAI has not been studied extensively, although WBC, CRP and Mcyt are routinely measured within the first hours of admission. Copeptin and PCT measured on admission were good predictors of any infection, pneumonia and UTI in the present cohort. They showed a similar predictive value for future infection compared to WBC and CRP. In a recent study neither WBC, CRP, Mcyt nor PCT measured on admission were sensitive enough to reliably be associated with SAI [18]. In another study, WBC and Mcyt count on admission did not differ between infected and non-infected stroke patients [19]. Only on day 1 after stroke onset, body temperature [18] and WBC [18,19] became significantly associated with infections after stroke. However, in these studies the time point of diagnosis in relation to biomarker measurements was not taken into account. Therefore, they could not really establish the predictive value of these markers but rather their diagnostic accuracy at the time of infection. Moreover the sample size was somewhat small and associations might have been missed due to lack of power. To our knowledge our study is the first to assess the predictive value of these markers taking into account the time point of measurements as well as diagnosis.

In the present study, each laboratory parameter remained a strong predictor after adjusting for NIHSS, age and CI and infarct localization. This is an unexpected finding because age and stroke severity may also contribute to SIS and thus infection after acute ischemic stroke [20–22]. However, these biomarkers seem to add prognostic information beyond age, stroke severity and a higher CI as well as infarct localization.

Copeptin was a strong predictor for SAI on admission and during the acute phase of stroke. The predictive value of copeptin

Table 4. OR to predict infections associated with nearest predictor measurements adjusted for age, NIHSS and CI as well as supra- or infratentorial infarct localization.

	OR (95%CI) adjusted for age	OR (95%CI) adjusted for NIHSS	OR (95%CI) adjusted for CI	OR (95%CI) adjusted for supra−/ infratentorial infarctions
Any Infection				
Temperature	2.36 (1.48–3.75)	2.10 (1.35–3.28)	2.82 (1.46–3.56)	2.30 (1.45–3.65)
PCT	1.64 (1.27–2.12)	1.62 (1.26–2.07)	1.81 (1.37–2.40)	1.69 (1.30–2.20)
CRP	2.23 (1.72–2.90)	1.96 (1.47–2.60)	2.22 (1.70–2.90)	2.28 (1.75–2.96)
WBC	4.97 (3.42–7.21)	4.22 (2.86–6.21)	4.90 (3.34–7.20)	4.80 (3.33–6.91)
Mcyt	1.69 (1.37–2.07)	1.70 (1.37–2.10)	1.68 (1.37–2.06)	1.72 (1.40–2.11)
Copeptin	2.22 (1.64–3.02)	1.84 (1.21–2.79)	2.30 (1.72–3.70)	2.43 (1.81–3.25)
Pneumonia				
Temperature	3.11 (1.23–7.86)	2.64 (1.11–6.29)	2.95 (1.23–7.09)	2.95 (1.24–7.00)
PCT	1.89 (1.33–2.67)	1.88 (1.33–2.65)	2.15 (1.40–3.32)	1.95 (1.37–2.79)
CRP	2.58 (1.79–3.71)	2.25 (1.48–3.42)	2.60 (1.77–3.80)	2.67(1.86–3.82)
WBC	4.17 (2.41–7.22)	3.73 (2.17–6.41)	4.32 (2.58–7.23)	4.30 (2.55–7.28)
Mcyt	2.09 (1.63–2.67)	2.13 (1.65–2.75)	2.15 (1.71–2.71)	2.19 (1.72–2.79)
Copeptin	3.07 (2.08–4.53)	2.95 (1.70–5.11)	3.28 (2.24–4.81)	3.37 (2.28–4.98)
Urinary Tract Infection				
Temperature	1.66 (0.78–3.55)	1.48 (0.76–2.88)	1.61 (0.76–3.42)	1.63 (0.78–3.42)
PCT	1.56 (1.16–2.10)	1.54 (1.12–2.11)	1.74 (1.19–2.53)	1.67 (1.21–2.29)
CRP	2.21 (1.49–3.29)	1.98 (1.31–3.00)	2.21 (1.45–3.36)	2.46 (1.64–3.69)
WBC	4.50 (2.82–7.18)	4.18 (2.48–7.06)	4.76 (2.75–8.25)	4.86 (2.99–7.92)
Mcyt	1.97 (1.56–2.49)	1.99 (1.56–2.53)	2.02 (1.48–2.77)	2.08 (1.63–2.67)
Copeptin	1.86 (1.20–2.89)	1.65 (0.85–3.20)	1.92 (1.19–3.09)	2.02 (1.32–3.10)
Other Infection				
Temperature	6.94 (2.52–19.12)	5.75 (2.10–15.71)	6.57 (2.50–17.29)	6.52 (2.23–19.06)
PCT	1.29 (0.93–1.78)	1.24 (0.87–1.77)	1.37 (0.97–1.92)	1.36 0.99–1.88)
CRP	2.25 (1.50–3.37)	1.91 (1.16–3.14)	2.30 (1.53–3.44)	2.44 (1.56–3.81)
WBC	5.54 (3.49–8.78)	5.01 (2.93–8.56)	5.62 (3.48–9.08)	6.08 (3.75–9.88)
Mcyt	1.32 (0.96–1.82)	1.30 (0.95–1.79)	1.33 (0.95–1.84)	1.34 (0.95–1.91)
Copeptin	2.28 (1.36–3.79)	1.60 (0.75–3.42)	2.37 (1.50–3.74)	2.17 (1.31–3.59)

PCT: procalcitonin; CRP: C-reactive protein; WBC: white blood cells; Mcyt: monocytes.
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in respect of SAI was similar to that of established biomarkers of infection (i.e. WBC, CRP). This finding might be due to the association of copeptin with the activation of the HPAA: increased copeptin-levels probably indicate a high degree of stress and SIS, which means a higher susceptibility to develop an infection. The prognostic value of PCT was also in the range of WBC and CRP. In the literature PCT is a superior diagnostic marker in pneumonia and other bacterial infections when compared to WBC and CRP [23]. However, the prognostic accuracy of a single PCT valueis limited [24]. PCT might be rather a specific than a sensitive prognostic marker in predicting infections.

The combination of established inflammatory makers (WBC, CRP) combined with a biomarker of stress, i.e. copeptin or a biomarker of bacterial infection, i.e. PCT [16] improves prediction of SAI compared to the strongest prognostic marker alone. The combination of biomarkers probably reflects better the complexity of an infection than one biomarker alone and may lead to a more accurate prediction of a beginning but not yet clinically apparent infection.

The investigated biomarkers seem to detect infections before clinical or paraclinical signs prompt further diagnostic work-up leading to the diagnosis of infection. Thus, these markers may help in risk stratification and may select high-risk patients for intervention studies.

We are aware of the following limitations: First, our results are based ona single cohort and our findings need to be validated in an independent and larger cohort. Second, the sample size was relatively small when assessing subgroups of infection. The bivariate analysis may have a limited statistical power and validity underestimating possible effects of biomarkers and other potential predictors. Third, although WBC and CRP was not a criterion for making the diagnosis of pneumonia, any infection and UTI, one must take into account that WBC was one of three criteria for the diagnosis of the subgroup of OI. Therefore, the good predictive value of WBC - in the case of OI - is most probably due to incorporation bias. This, on the other hand, strengthens the predictive value of copeptin that might be underestimated compared to WBC in this study. Fourth, we are not able to proof causalities or provide more insights into pathomechanisms, to explain why these markers are good predictors of infections even before clinical signs occur. But even if these markers are only surrogates of underlying processes which predispose patients for

Table 5. Comparison of AUCs for developing infection between the predictors WBC, Mcyt, CRP and Copeptin.

Variables	AUC	p-value
Any Infection		
WBC vs Mcyt	0.82 vs 0.65	<.001
WBC vs CRP	0.82 vs 0.74	0.16
WBC vs Copeptin	0.82 vs 0.75	0.07
CRP vs Copeptin	0.74 vs 0.75	0.75
CRP vs Mcyt	0.74 vs 0.65	0.04
Copeptin vs Mcyt	0.75 vs 0.65	0.05
Pneumonia		
WBC vs Mcyt	0.81 vs 0.72	0.13
WBC vs CRP	0.81 vs 0.80	0.78
WBC vs Copeptin	0.81 vs 0.86	0.72
CRP vs Copeptin	0.80 vs 0.86	0.98
CRP vs Mcyt	0.80 vs 0.72	0.36
Copeptin vs Mcyt	0.86 vs 0.72	0.28
Urinary Tract Infection		
WBC vs Mcyt	0.83 vs 0.69	0.09
WBC vs CRP	0.83 vs 0.74	0.24
WBC vs Copeptin	0.83 vs 0.71	0.14
CRP vs Copeptin	0.74 vs 0.71	0.86
CRP vs Mcyt	0.74 vs 0.69	0.64
Copeptin vs Mcyt	0.71 vs 0.69	0.68
Other Infection		
WBC vs Mcyt	0.84 vs 0.61	0.008
WBC vs CRP	0.84 vs 0.74	0.10
WBC vs Copeptin	0.84 vs 0.75	0.02
CRP vs Copeptin	0.74 vs 0.75	0.80
CRP vs Mcyt	0.74 vs 0.61	0.28
Copeptin vs Mcyt	0.75 vs 0.61	0.30

PCT: procalcitonin; CRP: C-reactive protein; WBC: white blood cells; Mcyt: monocytes.
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infections, from a clinical standpoint we belief that the observed associations are very interesting since we identified accurate prognostic markers for risk stratification. Finally, the distinction between prediction and early diagnosis of infection is difficult. We are not able to differentiate whether the biomarkers investigated in this study might rather detect infections at an early state or predict vulnerability for future post-stroke infections, although we excluded patients with possible infection prior to the onset of stroke.

In summary, copeptin, PCT, WBC and CRP were good predictors of the development of any infection, pneumonia and UTI. The combination of the 3 biomarkers even improved the prognostic value by accurately separating patients with and without future infections already on admission. If validated in larger prospective studies the combination of these 3 biomarkers with best AUC values may add significant information for the early identification of high-risk patients. Future intervention studies could select patients with high-risk profiles according to these biomarker levels and these high-risk patients may proof to benefit from prophylactic antibiotic treatment.

Table 6. Comparison of batches with best predictors of specific type of infection alone.

	Adjusted OR*	CI (95%)	p-value	AUC	Wald-p**
Batches 1: WBC+CRP+Copeptin					
Any infection					
WBC	3.70	2.26–6.08	<0.001	0.86	<0.001
CRP	1.66	1.24–2.21	<0.001		
Copeptin	1.53	1.07–2.18	0.019		
Pneumonia					
WBC	4.12	1.63–10.39	0.003	0.92	<0.001
CRP	1.92	1.30–2.84	0.001		
Copeptin	2.06	1.19–3.57	0.010		
Urinary Tract infection					
WBC	3.11	1.55–6.24	0.001	0.85	0.058
CRP	1.62	1.01–2.61	0.047		
Copeptin	1.26	0.73–2.19	0.411		
Other Infections					
WBC	6.84	3.00–15.60	<0.001	0.90	0.43
CRP	1.29	0.87–1.93	0.208		
Copeptin	1.18	0.69–2.03	0.550		
Batch 2: WBC+CRP+PCT					
Any infection					
WBC	3.67	2.42–5.58	<0.001	0.84	<0.001
CRP	1.56	1.16–2.11	0.003		
PCT	1.25	0.99–1.57	0.064		
Pneumonia					
WBC	4.25	2.27–7.97	<0.001	0.90	<0.001
CRP	1.87	1.39–2.52	<0.001		
PCT	1.36	1.00–1.85	0.052		
Urinary Tract Infection					
WBC	2.89	1.69–4.95	<0.001	0.82	0.014
CRP	1.59	0.90–2.81	0.114		
PCT	1.19	0.82–1.70	0.359		
Other Infections					
WBC	5.74	3.33–9.87	<0.001	0.89	0.25
CRP	1.53	0.92–2.55	0.103		
PCT	0.85	0.59–1.24	0.407		

WBC: white blood cells; CRP: C-reactive protein; PCT: procalcitonin. AUC: Area under the curve to predict infection using the combined model of all predictors.g.
*adjusted for all predictors in the respective model.
**Wald-p: refers to the comparison of the combined model with the model of the strongest predictor, alone which always was WBC.
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Author Contributions

Conceived and designed the experiments: FF MK. Performed the experiments: FF MK. Analyzed the data: FF MK BM MCC. Contributed reagents/materials/analysis tools: BM MCC NGM. Wrote the paper: FF

MK. assay development and measurement of copeptin and procalcitonin levels: NGM Supervision of the writing of the report: MCC, BM.

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